U. S. DEPARTMENT OF AGRICULTURE.
BUREAU OF ANIMAL INDUSTRY.
BULLETIN No. 3.

MISCELLANEOUS INVESTIGATIONS

CONCERNING

INFECTIOUS AND PARASITIC DISEASES

OF

DOMESTICATED ANIMALS.

CONDUCTED UNDER THE DIRECTION OF DR. D. E. SALMON,
CHIEF OF THE BUREAU OF ANIMAL INDUSTRY,

BY

F. L. KILBORNE, VERANUS A. MOORE, E C. SCHROEDER,
THEOBALD SMITH, AND C. W. STILES.

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LETTER OF TRANSMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY,
Washington, D. C., August 29, 1893.

SIR: I have the honor to herewith transmit the results of some important investigations concerning infectious and parasitic diseases, as revealed by researches made by my assistants in the laboratory and at the Experimental Station of the Bureau while engaged in efforts to determine the causes of some of the more destructive communicable and contagious diseases of domesticated animals.

Very respectfully,

D. E. SALMON,
Chief of Bureau.

Hon. J. STERLING MORTON,
Secretary of Agriculture.

LETTER OF SUBMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY,
Washington, D. C., April 25, 1893.

SIR: I have the honor to submit herewith some investigations concerning infectious and parasitic diseases of domesticated animals, which are either the outgrowth of more important researches already published or else fragments of such work under way. The slow progress made, largely due to the want of suitable material for investigation, makes it very desirable that integral portions of our work be not withheld too long from publication, provided premature conclusions are not drawn therefrom. The minor investigations herewith presented are all of them important in aiding our understanding of infectious animal diseases, and in paving the way for their control or suppression.

Very respectfully,

THEOBALD SMITH,
Chief of the Division of Animal Pathology.

Dr. D. E. SALMON,
Chief of the Bureau of Animal Industry.

MISCELLANEOUS INVESTIGATIONS CONCERNING INFECTIOUS AND PARASITIC DISEASES OF DOMESTICATED ANIMALS.

OBSERVATIONS ON THE MORPHOLOGY, BIOLOGY, AND PATHOGENIC PROPERTIES OF TWENTY-EIGHT STREPTOCOCCI FOUND IN THE INVESTIGATION OF ANIMAL DISEASES.

By VERANUS A. MOORE.

In the bacteriological investigation of diseased animal tissues which has been carried on in this laboratory, streptococci have frequently been found, either alone or associated with other bacteria. Their occasional appearance in greater or less numbers in the variously affected organs, more especially when associated with those morbid conditions which could not be attributed to any specific cause, suggested the desirability of determining as far as possible the relations existing between the streptococci and the lesions in which they were found. The results that had been obtained, and the observations that had been made by a few investigators with reference to the etiological value of certain streptococci, gave hope that with a better knowledge of this group of bacteria the cause of a greater or less number of morbid conditions might be explained. Since the summer of 1888, therefore, the streptococci that have appeared in the regular examinations in this laboratory have for the greater part been isolated and subjected to a somewhat thorough investigation with reference to their morphology, biology, and especially their pathogenic properties. It should be stated that several of the streptococci that had appeared prior to this time had been quite carefully studied, but no attempt had been made to isolate and compare all of these forms. My work upon this group of bacteria has been done under the direction of Dr. Theobald Smith, Director of the Laboratory, to whom I am indebted for valuable suggestions and assistance in the isolation of many of these streptococci. For the inoculation of pigs and the careful watching of the same subsequently, I am indebted to Dr. F. L. Kilborne. Other and more important work has necessarily confined this study within quite narrow limits, and prevented the carrying out of extended experiments in any of the many directions suggested by these preliminary observations.

Since Billroth called attention to this group of bacteria, much has been written concerning the streptococci that have been found to be associated with lesions in a large number of diseases in both man and the lower animals. A few of these streptococci have been found by careful study and investigation to be of specific etiological value, but a considerable number of them, on account of their frequent appearance and their pathogenic effect on certain of the experimental animals, have been considered as bearing a more or less causal relation to the lesions with which they were associated. While it is my purpose to present simply the results of the observations made on the streptococci examined, a brief mention of the more important work that has been done on this subject, and from which many suggestions have been obtained, should not be omitted.

Koch found in putrid fluids a streptococcus which, when inoculated into the ear of a mouse, produced necrosis of the surrounding tissue, destruction of the cartilage cells, and death of the animal in about three days.

In the earlier investigations of swine diseases carried on by the U. S. Department of Agriculture, Dr. Salmon isolated micrococci from the diseased organs of pigs. The micrococci grew in shorter or longer chains, and in stained cover-glass preparations they exhibited a light center. These forms were so constantly present that they were supposed to have a more or less causal relation to the diseases with which they were associated.

Klein, in his investigation of a cattle disease known as "cow scarlatina," or the "Hendon cow disease," found a streptococcus in the sores on the udders of the diseased cows. He isolated and carefully studied this streptococcus. When it was inoculated into calves and mice it produced a definite disease, which he considered to be identical with the disease that was produced by the scarlatina streptococcus. In another outbreak, which manifested itself by a contagious, ulcerative process on the teats and udders of certain cows which furnished milk to the Edinburgh dairy, other streptococci were obtained, one of which Klein considered as standing in some "necessary relation to the disease."

The same author, in his investigations of "foot-and-mouth disease" in sheep, concludes that the cause of the disease is a micrococcus, which grew in chains. He obtained the germ from cultures made from the lymph of a sheep which was affected with the disease. Inoculation experiments with this germ were negative, but by feeding sheep with cultures of the twentieth generation the typical disease was produced. From the vesicles of the artificially-produced disease pure cultures of the micrococcus were obtained.

Sand and Jensen in 1887 investigated the cause of strangles in horses. In 29 cases they invariably found in the pus from the suppurating glands and in the nasal discharge a characteristic streptococcus.

It grew on all of the ordinary culture media. It destroyed mice, and two of the three rabbits that were inoculated with it in the veins died. Guinea-pigs were immune. Horses were not affected, but colts developed typical strangles after spraying the nasal mucous membrane with a bouillon culture of the streptococcus. One of these died. Two colts that were inoculated intravenously suffered from phlebitis and abscesses, after which they were immune.

Schütz found a streptococcus in the pus from the glands of horses suffering from strangles. It was fatal to mice, but rabbits, guinea-pigs, and pigeons were immune. The nasal cavities of a colt, 6 to 8 weeks old, were sprayed several times with a pure bouillon culture of this germ. It died of strangles on the 23d day. Schütz examined the pus from the suppurating glands of a considerable number of horses suffering from strangles, in all of which he found the streptococcus. Zschokke, in 1886, found a streptococcus in horses suffering from strangles, but supposed it, prior to Schütz's investigations, to be identical with *Streptococcus pyogenes.*

In addition to these observations it is a fact worthy of note that streptococci are frequently found associated with a considerable number of morbid conditions which thus far have not been satisfactorily attributed to any specific germ.

In human pathology the presence of streptococci is not unusual. The etiological value of *Streptococcus pyogenes* Ogston, and *Streptococcus erysipelatos* Fehleisen, is very generally conceded. Such affections as erysipelas, pyæmia, empyema, septicæmia, diphtheria, progressive gangrene, scarlatina, Impetigo contagiosa, pericarditis, ostemyelitis, and influenza have been attributed with more or less positive evidence to the invasion of certain streptococci. The investigations of the etiology of la grippe has revealed the fact that streptococci are very frequently associated with the inflammatory condition of the air passages in that disease. Prudden has made the observation that streptococci are found associated, apparently as the etiological factor, with those inflammations, particularly of the serous membranes, in which the formation of fibrin is a most marked feature.

Investigators are not unanimous in the belief that streptococci which have been found to be associated with the diseases mentioned belong to different species. It is a well known fact that their common morphological and biological properties are much more numerous than their differences, and that it is exceedingly difficult to point out a distinguishing trait of any one of these streptococci that is sufficiently characteristic to differentiate it from the others.

The description by Flügge of *Streptococcus pyogenes* applies equally as well to *Streptococcus erysipelatos*, and also to a large number of other streptococci which have no pathogenic properties by which they can be differentiated. Hajek could not find any morphological or cultural difference between *Streptococcus erysipelatos* and *Streptococcus pyogenes*,

but by inoculation experiments he found that they possessed marked differences.

Prudden compared very carefully *Streptococcus pyogenes*, *Streptococcus erysipelatos*, and *Streptococcus diphtheriæ*, but found that there was not a single constant feature of difference between them that could be detected by cultural characters (parallel cultures), or by inoculations into the smaller experimental animals.

Fraenkel isolated from a case of purulent peritonitis a streptococcus which had apparently played the part of a pyogenic agent, and succeeded in producing erysipelas on the ear of a rabbit with a pure culture of this streptococcus. When it was inoculated beneath the skin on the back of mice and rabbits it produced suppuration.

Prof. Welch was unable to determine any decisive differences between *Streptococcus erysipelatos* and *Streptococcus pyogenes*. He adds that "the efforts to differentiate into distinct species the pathogenic streptococci have thus far met with little success, so that the weight of opinion favors the view that the streptococci of erysipelas, phlegmonous inflammations, septicæmia, puerperal fever, and of the various forms of angina belong to one and the same species."

Klein, however, in his report on "The Morphology and Biology of the Streptococcus," in which he discusses the properties of nine selected streptococci, concludes that they are separate and belong to distinct species or varieties. He also believes that a large number of streptococci that have the appearance, on a superficial examination, of belonging to the same species, may, by careful study, be differentiated into separate species or varieties.

Von Lingelsheim has recently divided the streptococci into two groups; one non-pathogenic, called *Streptococcus brevis*, and the other pathogenic, called *Streptococcus longus*. The streptococci which he studied were from various sources, principally, however, from the normal human saliva and diphtheritic membranes. He made some interesting investigations upon the effect of disinfectants on the various streptococci which he studied. He lays much stress upon the importance of blood serum from various animals as a culture medium for these germs. His streptococci grew very vigorously on the serum from rabbit's blood, while they failed, with one exception, to grow on that from pig's blood.

Kurth, in his extensive work upon the differentiation of streptococci and their appearance, especially *Streptococcus conglomeratus* in scarlatina, makes the following provisional classification. *Streptococci rigidi*, which grow in short chains in bouillon, the sediment loose, the chains not hanging together. *Streptococci flexuosi*, which grow in long chains in bouillon, the sediment hanging together.

Pasquale, in an exhaustive discussion of the greater number of the streptococci that have heretofore been described, has tabulated the various properties of 33 different species, making note of their effects on milk, acid formations, pigment-producing properties, growths upon

various kinds of blood serum, and their pathogenic effects. Their morphology and cultural characters on the ordinary media are also carefully considered. The large variety of species with which he has worked has enabled him to take a more comprehensive view of the entire group of streptococci than had heretofore been done. The result of his study of the pathogenic powers of the different species of streptococci differs in many respects from those obtained by other authors. He has, as a result of his observations, formulated a new classification of this group of bacteria based upon their morphological and pathogenic properties. He does not, however, draw sharp lines between the different streptococci, but on the contrary groups them within the natural boundaries which he finds to exist between them.

Pasquale's classification of the streptococci is as follows:

I. SHORT SAPROPHYTIC STREPTOCOCCI.
   (1) Develop in a low temperature (Feces and external surroundings).
   (2) Develop in a higher temperature (Mouth and bronchial mucosa).

II. LONG, NOT VIRULENT, STREPTOCOCCI.
   (1) Feces. (Example, *Streptococcus coli gracilis.*)
   (2) Mucous membrane of the mouth (Strepococcus of Kruse u. Pansini).

III. LONG PATHOGENIC STREPTOCOCCI.
   (1) Streptococci of erysipelas, suppuration, pneumonia, diphtheria, scarlatina, etc.
   (2) Sputum of pneumonia (Streptococcus of Kruse u. Pansini).

IV. SHORT, HIGHLY INFECTIOUS, STREPTOCOCCI.
   (1) From tuberculosis, etc. (*Diplococcus pyogenes*).
   (2) From pneumonia (*Diplococcus pneumonia*).

It is believed that the investigations cited are sufficient to show the general scope of the work that has been done and the more important opinions entertained concerning this very interesting class of the shizomycetes, although a very long list of minor observations could be added. Without entering into detail, it may be stated that subsequent investigations have shown that in some cases, especially in human pathology, the streptococcus which was first supposed to be the etiological factor in the disease gave way to other bacteria* and in a few other cases the question is still an open one. With this brief review I will pass to the consideration of the streptococci that have come under my observation. It should be stated at the outset that the streptococci which are here considered were obtained exclusively from the organs and secretions of domesticated animals, and that no attempt is made to identify them with the streptococci that have heretofore been described. This is omitted on account of the great similarity,

**Streptococcus diphtheriæ* is a marked example of this fact. Prudden found this streptococcus almost invariably in the false membrane and occasionally in the spleen, liver, and kidneys in a series of twenty-four cases in children who perished with the disease. In none of these did he find the Klebs-Loeffler bacillus. In a later series of investigations, however, he found the Klebs-Loeffler bacillus in every case.

generally speaking, that exists in the morphological and biological characters between the streptococci isolated from different sources and the variability of their cultural characters when grown under different conditions.

The first streptococcus to which my attention was given was discovered by Dr. Theobald Smith, in the spring of 1887, while he was investigating an outbreak of swine plague.* Since that time several other outbreaks of swine disease have been investigated, in each of which one or more streptococci have been isolated from the diseased organs of one or more of the pigs that were examined. In addition to these, streptococci have been isolated from diseased organs taken from animals that died from varied and widely separated diseases. They have also been found to to be quite numerous in the secretions covering the different mucuous membranes of the animal body as well as in various extraneous material. This varied, and we may almost say, universal distribution of these organisms, renders it of much importance in view of the great similarity that is known to exist between them, that differential biological or morphological characters should be determined, if they exist, between the purely saprophytic streptococci and those that have a greater or less etiological value.

In all we have isolated during the past four years more than fifty streptococci, many of which perished before they were sufficiently studied to be considered here. In a few of the earlier cases streptococci, which appeared upon a somewhat superficial examination to be identical, were found in several organs of the same animal, and consequently only one culture was retained for further study and comparison. This was unfortunate, as subsequently it was found in a few cases that the streptococci that were present in different parts of the body could be differentiated by one or more distinct characteristics. A few of the streptococci were obtained in pure culture from the diseased organs of the animals that were examined, while others were isolated from impure original cultures by means of agar plates. The germs thus obtained have been carefully studied and compared microscopically and in their growth upon certain culture media. Various experimental animals have been inoculated with pure cultures of these germs, and subsequently very carefully watched in order to detect any effect that might have been produced by the inoculation. The greater number of the streptococci considered here were isolated from morbid tissues, but for the purpose of comparison a few that were obtained from the mucous membranes of certain animals are inserted.

The cultivation of these streptococci has shown them to be very sensitive to any change in the conditions under which they were grown, especially to any variation in the composition or chemical reaction of the culture media. On account of this extreme delicacy in the great majority of their differential biological characters, it seems best at

*This streptococcus was described by Dr. Smith in the Bureau Report for 1887.

this time to limit the descriptions of these characters to those more hardy properties which repeated cultures have shown to be constant, and consequently recognizable under slightly varying conditions as having a more or less differential value. It will be observed subsequently that the greater number of constant differences that exist between these streptococci were manifested in their physiological effect, and it is believed by the writer that when our methods for the determination of these properties are sufficiently elaborated the facts necessary for a specific differentiation of the various classes of the schizomycetes will be forthcoming.

Although several classifications of the streptococci have been made, there appears to be no system of subdivision which is applicable to all of the series of streptococci which have been described. My observations have shown that there are streptococci which possess biological characters that give them an intermediate position between the forms which have been designated as belonging to subgroups. On this account I have not adopted any of the classifications which have been formulated. The streptococci which I have studied, as the subsequent descriptions will show, exhibit a more or less marked variation in their various properties, but, with one exception, they were as sensitive to changing conditions of growth as *Streptococcus erysipelatous* and other recognized pathogenic forms. It would seem, therefore, that many of these forms have become parasitic in their habits. A classification based on the various morphological, biological, or pathogenic properties of a limited number of streptococci must continue to suffer modifications as more extended observations are made, until the saprophytic, parasitic, and pathogenic forms are more clearly defined, and the range of their biological variations more accurately determined.

Before considering the special qualities of the streptococci about to be described, a few of the more important properties which have been found to be common to them all may be mentioned:

1. They are readily stained with the ordinary aniline dyes.
2. They grow in gelatine, but do not liquefy it.
3. The growth on the different media is not viscid.
4. The growth at the ordinary temperature is less vigorous than at the temperature of the body (excepting streptococcus O).
5. In simple beef broth the growth is more feeble than in peptonized bouillon.
6. The growth is most vigorous in peptonized bouillon containing glucose.
7. With one exception (streptococcus O) the time during which cultures will remain alive, is very variable and uncertain. (It is due to this fact that several forms were lost before their characteristics were determined.)

In order to present, in a form most convenient for comparison, a description of the more important properties of the streptococci studied the following table has been constructed:

Description of the

Strepto-cocus.	Source and date.	Accompanying disease.	Length of chains.	Diameter of cocci.	Growth on solid media.		
					Agar.	Gelatine.	Potato.
A	Lung, pig, 1887....	Swine plague.	Very long.	0.8 μ oval.	.....	Feeble...	No growth.
B	Spleen, pig, 1888 ..	Disease (?) ...	Long	0.6–0.9 μ ..	Nuclear†..	...do	Grayish ...
C	Lung, pig, 1889....	Hog cholera ..	Short and long.	0.7–0.9 μ ..	...do	...do	.....
D	....do	....do	...do	0.7–0.9 μ ..	...do	...do	.....
E	Spleen, pig, 1889 ..	....do	Long and very long.	0.5–0.7 μ ..	...do	...do	No growth.
F	....do	Modified hog cholera.	...do	0.7–0.8 μ ..	...do‡	...do	Thin, grayish.
G	....do	Disease (?) ...	Very long.	0.8–1.0 μ ..	...do	...do	Feeble.....
H	Lung, pig, 1889....	....do	Short	0.7–0.9 μ ..	...do	...do	.....
I	....do	Swine plague.	Long	0.6–0.7 μ ..	...do	...do	No growth.
J	Blood, pig, 1889....	Modified hog cholera.	...do	0.6–0.7 μ ..	...do	...do	Feeble.....
K	Peritoneal exudate, pig, 1890.	Swine plague.	...do	0.6–0.8 μ ..	...do§	...do	.....
L	Liver, pig, 1890....	....do	...do	0.8–1.8 μ ..	...do	...do	.....
M	Spleen, pig, 1890 ..	Disease (?) ...	...do	1.0–1.5 μ ..	...do‡	...do	No growth.
N	Kidney, cow, 1890.	Texas fever...	Short	0.8–1.0 μ ..	...do	...do	.....
O	Feces, cow, 1890 ...	Healthy	...do	0.8–1.8 μ ..	Vigorous .	Vigorous	.....
P	Trachea, pig, 1890.	....do	...do	0.8–1.0 μ ..	Nuclear ..	Moderate	.....
Q	Lung, cow, 1891 ...	Pneumonia ...	Long	1.2–2.0 μ ..	Moderate.	Feeble...	.....
R	Abscess, knee, pig, 1891.	Chronic hog cholera.	Short	0.7–0.8 μ ..	...do	...do	.....
S	Abscess, elbow, pig, 1891.	....do	...do	0.7–0.8 μ ..	...do	...do	.....
T	Lung, cow, 1891 ...	Pleuro-pneumonia.	...do	1.0–2.0 μ ..	...do	...do	No growth.
U	Trachea, horse, 1891.	Healthy (?)...	...do	0.9–1.0 μ ..	Translucent.	...do	Feeble.....
V	Lung, pig, 1891....	Swine plague.	Long	0.7–0.8 μ ..	Moderate .	...do	No growth.
W	Liver, pig, 1892....	Disease (?) ...	...do	0.8–0.9 μ ..	Nuclear...	...do	...do .....
X	Blood, pig, 1892 ...	....do	Short	0.7–0.9 μ ..	...do	...do	...do .....
Y	Spleen, pig, 1892 ..	....do	Long	0.7–0.8 μ ..	...do	...do	...do .....
Z	Liver, pig, 1892....	....do	Short	0.8–1.3 μ ..	Moderate .	...do	...do .....
α	Blood, pig, 1892 ...	....do	Long	0.7–0.9 μ ..	...do	...do	...do .....
β	Nasal mucus, horse, 1892.	Glanders.....	Short	0.9–1.0 μ ..	Nuclear ..	...do	...do .....

*The streptococci from Q to β inclusive were cultivated in peptonized bouillin containing 2 per cent glucose in the fomentation tube. Streptococci Q, R, and S, did not develop in the closed portion of the tube. The growth in the open bulb was exceedingly vigorous. The others developed in both sides of the tube. The growth was more vigorous but otherwise similar to that in simple bouillon. The alkaline reaction of the bouillon was changed in every case to an acid one during their multiplication.

†There was no formation of pigment in the growth on the surface of agar.

‡An unusually vigorous growth.

§An unusually feeble growth.

‖Several weeks.

twenty-eighth streptococci.

Growth in bouillon.*	Reaction.	Bouillon cleared.	Effect on milk.	Stain after—		Pathogenesis.
				Gram's method.	Weigert-Gram method.	
		Days.				
Flakes			Not changed	Nicely		Destroys mice.
F'ntly cl'd'd, flakes.		10	Thickened in 30 days.	Not stained.	Not stained.	Local swelling in rabbits.
Uniformly clouded.		26	No change	Nicely	Feebly	No effect.
do		19	do	do	do	Do.
Flakes, faintly clouded.		12	Casein coagulated.	Feebly	do	Destroys mice and rabbits.
Uniformly clouded.		25	No change	Nicely	Nicely	No effect.
Flakes in suspension.		12	do	Feebly	do	Do.
Uniformly clouded.		10	do	do	do	Do.
do		3	Casein coagulated.	Nicely	do	Do.
do		40	Thickened	do	Feebly	Do.
Flaky sediment.			No change	Not stained.	Very feebly.	In rabbit elevation of temperature. Local swelling.
do			Casein coagulated.	do	Feebly	No effect.
Heavily cl'd'ed, flakes in suspension.		6	No change	Nicely	Nicely	Do.
Turbid		(‖)	do	do	Feebly	Do.
do		15	Casein coagulated.	Not stained.	Not stained.	Do.
F'tly cloud'd.		8	do	Feebly	Nicely	Do.
Flocculent sediment.	Acid*		No change	do	Feebly	Slight local reaction in rabbits.
Flakes	do		Casein coagulated	do	Nicely	No effect.
do	do		No change	Nicely	Not stained.	Do.
Uniformly clouded.	Alkaline	8	Casein coagulated.	do	Nicely	Destroys mice and rabbits.
do	Acid	4	do	Feebly	Not stained.	Do.
do	Alkaline	7	do	Nicely	Nicely	No effect.
Ft'ly clouded, flakes.	Acid	5	do	Feebly	Not stained.	Do.
do	do	2	do	Nicely	Nicely	Do.
do	do	4	No change	do	do	Do.
do	do	2	do	do	Not stained.	Destroys mice.
do	do	2	do	do	Nicely	Destroys mice, rabbits, and pigs.
Very faintly clouded.	Alkaline	6–8	do	do	do	No effect.

* According to observations recently made by Dr. Theobald Smith, the initial acidity of the cultures of many bacteria is demonstrably due to the presence of muscle-glucose in the bouillon. As this varies in quantity and is occasionally absent, the inconstant and conflicting results on the acid and alkaline reaction of cultures is readily explained.

A few explanations seem necessary, however, for an understanding of the terms employed in tabulating these facts:

1. In discussing these streptococci it was necessary to assign to each some name by which it could be designated. For this purpose the letters of the alphabet were chosen.

2. In the beginning of our work several of the tests were not employed that have been used in studying the forms more recently isolated.

3. The streptococci are given in the order of their discovery.

4. The words short, long, etc., used in designating the length of the chains, have been assigned the following arbitrary definite meaning: Very short chains are composed of from 3 to 4 cocci or segments; short chains of from 4 to 10 cocci; long chains of from 10 to 40 cocci; very long chains of more than 40 cocci. In the table these terms have reference to the growth in bouillon only. In cultures containing chains ranging from 3 to 50 or more cocci, the term has been employed that applies to the greatest number of individuals.

5. The media used were faintly alkaline in reaction. The agar, gelatine, and bouillon contained one-quarter per cent peptone and one-half per cent sodium chloride. Bouillon used in the fermentation tubes contained, in addition, 2 per cent glucose. The word "nuclear" is used to designate colonies from 1 to 2 millimeters in diameter (on the inclined surface of agar) which have a convex, grayish, opaque center, with a spreading translucent border. The word "moderate" represents a corresponding homogenous non-nuclear growth. In the growth in gelatine "feeble" indicates the development of colonies varying from one-eighth to three-fourths millimetre in diameter.

The fermentation tube* enables us to determine approximately the aërobic and anaërobic properties of the bacteria; as the liquid in the closed bulb is deprived of its air during the sterilization, all bacteria that grow in it can be considered as more or less anaërobic. The glucose was added in order to detect the power of the various streptococci to cause the fermentation of sugar with or without the production of gas.

BIOLOGY.

In addition to the media mentioned, glycerine agar, blood serum, agar-gelatine, glycerinated bouillon, acid bouillon, and potato broth have been employed in the cultivation of these streptococci. Although von Lingelsheim found blood serum very advantageous, my experience with the streptococci in question showed this medium to be quite unsatisfactory. (I have used the serum from cattle only.) The growth was variable on the various other solid media and in the bouillon con-

*Dr. Theobald Smith called attention to the value of the fermentation tube in bacteriological work in 1890. Centralblatt f. Bacteriologie, Bd. VII, p. 502. It is figured and briefly described by the same author in his Report on the Cause and Prevention of Swine Plague. U. S. Department of Agriculture, 1891, p. 81.

taining glycerine. Like the swine-plague germ, the great majority of these streptococci would not grow in acid bouillon, although many of them would change the reaction of the alkaline medium to an acid one during their multiplication.

The character of the growth in bouillon is interesting. With few exceptions it will be observed that the long-chained streptococci grew in flakes, while those composed of short chains imparted a uniform cloudiness to the liquid. This property was constant. Streptococcus Q was carried through a series of 20 subcultures made at short intervals with no alteration in the character of the growth. Shorter series were made with several of the other streptococci with the same result. In a few cases, there was a faintly clouded appearance of the liquid in which the streptococci grew very largely in flaky masses. The flakes are due to the interlacing of the chains, presumably during the process of multiplication. The time required for the liquid to become clear was very constant (as tested by several cultures), although it varied very much with the different species (?). The settling of the streptococci did not indicate their death, but the limit of their growth in the liquid. The sediment was not viscid.

Although the growth on agar and gelatine varied slightly, the constant characters were very similar, and, with few exceptions, these cultures could not be considered of any differential value. Their growth on potato, their ærobic and anaerobic powers, and their effect on the reaction of bouillon and the casein of milk show more marked differences. Although with every germ tested the reaction of the alkaline bouillon containing glucose was changed to a very acid one, no gas was produced. These facts are significant in showing the differential importance of the physiological properties of these bacteria.

In considering the minor differences that exist between these streptococci, especially those that were isolated from animal tissues, it should be remembered that they were obtained presumably at different periods of time after their invasion, and that there is no positive information by which we can determine the changes produced in their various properties by the influence of their new environments and the variable time in which they were subjected to them.

MORPHOLOGY.

When examined in a fresh condition, (hanging drop) with a magnification of 500 diameters, the chains appear, as a rule, to be made up of micrococci 0.7 to 1.8 μ in diameter. When stained with methylene blue, or fuchsin, they appear as deeply stained oval or spherical bodies. With a magnification of 1,000 diameters, the cocci, in the majority of the forms, appear to be more or less elongated. Many of these exhibit the polar stain or belted appearance quite characteristic of the swine plague group of bacteria, while others show simply a light unstained center. The spherical forms usually appear as deeply stained bodies.

In many species (?) there seems to be a tendency of the segments to be closely united in pairs. This is believed to be due to the incomplete division of the cocci. The Gram and Weigert-Gram staining methods can not be considered of much importance as means of differentiation, on account of the variable degree to which the stain is retained by the different species. The individual deviations from the typical streptococcus (micrococci united in chains) are as follows:

In streptococci A, F, G, O, Q, and W, the individual segments were oval, elongated, and resembled somewhat, in a stained specimen, the appearance of the swine plague organism. In G, the long diameter appeared to be transverse to the long axis of the chain. An examination of a properly stained specimen, magnified 1,000 diameters, revealed the fact that a single segment was composed of two of the supposed cocci separated by an unstained band. The ends were nearly square, giving them the appearance of minute rectangular parallelograms. In streptococci B, E, M, and U, the terminal cocci, and frequently one or two within the chains, were much larger than the other segments. When only two were present they were invariably at the extremities. They were never observed in the other streptococci, and their significance is still somewhat speculative. Streptococcus L presented a great variation in the size of the cocci and length of the chains. So varied was the size that it was thought for a considerable time that it was an impure culture. Repeated plate cultures, however, developed only one form of colonies which were composed of streptococci of a similar character. The shorter chains were composed of the larger cocci and the longer ones of the smaller cocci. This variation* was also present, but to a less degree, in streptococcus Z.

Streptococcus O varied very considerably when grown in different media. In bouillon the segments were spherical, while on agar and gelatine they were oval. The morphological as well as biological characters of this streptococcus indicated its close relationship to the ordinary saprophytic micrococci. The length of the chains in nearly all of the species varied with the media; usually they were longer in bouillon than on solid media, and longest in bouillion containing glucose.

Although considerable variation was found in the length of the chains, and in a few cases in the size of the individual cocci, there was no appreciable change produced by artificial cultivation in the morphology of any of these forms. Streptococcus Q was carried through a long series of cultures in a liquid containing very little nutriment (0.5 cc. of bouillon and 9.5 cc. of sterile distilled water). The number of chains was greatly diminished, but no appreciable change was produced in the morphology of the individual chains or cocci. Cultures in bouillon containing 1 per cent peptone gave similar results. It is

* Koplik and Van Arsdale have recently noted the variation in the diameter of the cocci, in the different chains of the streptococcus found by them in diseased joints (osteomyelitis).

interesting to note in this connection that in the pathogenic forms the chains were very short, and often single cocci appeared in cover-glass preparations from the organs of the dead animals. The few forms that were fatal to experimental animals possessed no morphological or biological peculiarities that would suggest their pathogenic properties, or separate them as a class or group from the others. The parasitic tendency in the cultural characters of all the streptococci (excepting O) was very marked.

It is observable that, while there are many minor differences, there is still a strong similarity existing between the different streptococci mentioned (excluding streptococcus O), and that the difference in the species of the host animal or the disease with which they were associated was not marked by any distinct characteristic of the invading streptococci. It will be observed from the table that in the differential tests no two of the streptococci that were studied gave precisely the same reaction throughout, but each streptococcus differed in some respect from all of the others. This fact appears to support Klein's statement that there is a specific or at least variatal difference existing between the streptococci (isolated from different sources) which appear in a superficial examination to be identical. In many cases, however, the variation was so slight that it seems possible to find its cause in external conditions rather than in the inherent properties of the germ.

The statements at hand concerning the variations in the morphology, biology, and pathogenic properties of certain bacteria as found in nature, and the power to modify to a greater or less degree these properties in certain species by cultivating them under different conditions, would warrant the supposition that many of the minor differences in these streptococci could have been brought about by the different influences to which they had been subjected prior to their isolation. The close resemblance of the streptococcus from the tracheal mucus of a pig (streptococcus P), to those found within the organs of diseased swine is at least suggestive in tracing the source of these organisms. The fact that intestinal and tracheal bacteria invade the inner organs and cavities of the body is so well established that it is not difficult to believe that streptococci, known to be more or less numerous in the flora of the mucous membranes of the healthy animal, should occasionally, under favorable conditions, make their way into the various organs of their host.

A few very interesting differences in the reaction of streptococci isolated from different organs of the same animal have been observed. Streptococci G and H were isolated from the liver and lung respectively of a pig that presented the lesions on post-mortem examination of a somewhat modified case of swine plague. In sections of the lung streptococcus H was seen winding in and out among the cells in the alveoli. As there was considerable lung disease but no swine plague

or hog cholera bacteria discovered, the pathogenesis of this germ was thoroughly tested on mice and rabbits, but with negative results. Streptococcus G grew without exception in the longest chains of any form studied, but like streptococcus H it produced no effect on animals, the difference between them existing in their morphology and biology.

Streptococci R and S were isolated from the pus in the joint abscesses (knee and elbow) of a pig that died of chronic hog cholera. (The hog-cholera bacilli were obtained from the same abscesses). As they were isolated from the abscesses, produced presumably by the same cause in the same animal, it is evident that they were subjected to practically the same conditions after their invasion. They were not distinguishable excepting in their reaction to Gram's and Weigert-Gram's staining processes and in their effect upon milk, where repeated experiments proved conclusively that streptococcus R would invariably coagulate the casein, while streptococcus S produced no appreciable change. The constancy in their reaction to the staining reagents was likewise determined. A third and similar illustration of this peculiar variation of streptococci inhabiting the same host and apparently identical in the majority of their characteristics is found in streptococci Z and α. These were obtained from the same animal and differed principally in their virulence, the one being fatal to mice only, the other destroying mice, rabbits and swine.

Pasquale makes the remark that streptococci differing in their characters were isolated from different organs of the same animal.

OTHER STREPTOCOCCI.

The streptococci that perished before they were studied sufficiently to be considered here did not, as far as they were observed, present any characteristics that were markedly different from those here described. A large number of them were inoculated into mice or rabbits, or both, but always with negative results. There are, however, several streptococci that have been isolated and studied more or less thoroughly, which have not been included in the preceding series, but which appear to be of sufficient importance in throwing light upon the distribution of the delicate and more saprophytic forms of streptococci to be briefly considered. A glance at the table will show that streptococcus O (isolated from the feces of a cow) differs very decidedly from the other forms in its biological characters, and that in every way it was more saprophytic in its tendencies. Several apparently similar streptococci have been isolated, which, upon subsequent cultivation, lost their power to grow in chains and appeared as ordinary saprophytic micrococci or diplococci. Some of these only softened gelatine, while others liquefied it. I will describe briefly two of these supposed streptococci:

(1) *April 4, 1890.*—I examined a rabbit which had a large and apparently closed abscess in one of its nasal cavities. From the pus in this abscess I obtained a strep-

tococcus. It grew in short chains, and was composed of micrococci 1.2 to 1.5 μ in diameter. It stained nicely after Gram's method, and grew very vigorously on the ordinary media. After about four generations, however, it ceased to grow in chains, and appeared subsequently as simply micrococci.

(2) From the tracheal mucus of a sheep a streptococcus was isolated by means of gelatine plates. It consisted of micrococci 1.2 to 1.8 μ in diameter, united in short chains. It took the Gram stain and grew very vigorously on all of the ordinary media. After a few generations very few chains were observed, but diplococci and single germs appeared in large numbers. It liquefied gelatine. The variations in the size of the cocci is especially worthy of note.

During an investigation of an outbreak of abortion in mares, cultures were made from the vaginal secretion of five healthy animals, from four of which a streptococcus which grew in long chains was obtained, which, in the minuteness of its structure and delicacy of its cultural characters, surpassed any of those described in this article. The streptococci from the different animals appeared to be identical in their microscopic appearance and growth on certain media. They were cultivated only with difficulty. In the examination of Potomac river water and a series of soil examinations various streptococci were found, some of which, upon superficial examination, presented characteristics of strongly saprophytic bacteria, while others were parasitic in their tendencies. Dr. Smith gave me a culture of a streptococcus isolated by him from drinking water that was biologically as parasitic in its tendencies as any of those obtained from the organs of diseased animals.

Although these observations on streptococci from extraneous material could be multiplied, the facts stated are sufficient to show the wide variations found to exist in this group of bacteria, and that those forms which we have isolated from the organs of diseased animals do not possess, biologically, parasitic tendencies that are not equally as well marked in some of the streptococci found apparently in their normal habitat in nature. These facts are of much importance in considering the economic value of this group of bacteria.

PATHOGENIC PROPERTIES.

It will be observed from the table that fifteen of the streptococci were obtained from the organs of animals that had perished from a known disease, while nine of them were isolated from animals that were either healthy or that had died from a disease the specific cause of which was not positively determined. These facts are important in their bearing upon the economic value of these forms. Of the twenty-eight streptococci described, six were fatal to some one or more of the experimental animals. The source of these destructive forms shows that two of them were associated with specific disease germs; two were found in a pig in which no disease germ was discovered, although the pig was from a herd in which several of the animals had succumbed to hog cholera: one from a pleuro-pneumonia lung, and the other was

from the tracheal mucus of a lame, but otherwise healthy horse. In addition to these, three of the forms produced more or less local reaction in rabbits, but apparently had no effect on mice. Of these, two were associated with specific pathogenic bacteria, and the third was found in the organs of an animal that perished from an undetermined disease. The other nineteen forms exhibited no pathogenic properties whatever. In addition to the usual subcutaneous and intravenous inoculations several of them were injected in large quantities into the pleural and abdominal cavities of rabbits with no appreciable effect. It should be observed that six of the nine more or less pathogenic forms grew in long chains, while in the other three the chains were short.

Besides the fact that 79 per cent of these streptococci, and all of a considerable number of other forms isolated from similar sources, failed to destroy experimental animals, we should note a few other general facts which are important in considering the pro and con of their etiological value.

1. The streptococci were isolated from a very large variety of lesions, from many of which the specific germ was obtained.

2. Comparatively speaking, a small minority of apparently similarly affected organs contained streptococci.

3. The streptococci isolated, even those from undoubtedly the same kind of lesions, were not identical as indicated by the various differential tests applied.

In these respects my observations have differed from those of Fehleisen, Klein, Schütz, and others who have attributed specific etiological value to certain of the streptococci which they have studied. It will be remembered that in their investigations the same streptococci were found to be nearly or quite universally present in a greater or less number of similarly diseased conditions.

As my observations and results do not conform with the necessary requirements to establish a specific pathogenesis for a micro-organism, it would seem that the streptococci here described (that were obtained from animal tissues) must be considered as having accidentally* invaded the organs from which they were isolated. Their etiological effect is therefore reduced to a secondary place, which assures them of no greater economic value than that possessed by a large number of other bacteria that are frequently found in diseased animal tissues. A more detailed account of the inoculation experiments with the nine pathogenic forms is appended:

Streptococcus A.—For the pathogenic effect of this streptococcus I quote from the Report of the Bureau of Animal Industry for 1887: "Two rabbits and two mice were inoculated subcutaneously with one-twelfth cc. each of a pure bouillon culture. Both

*By the term "accidentally" is meant a passive invasion, brought about by conditions produced by other causes in contradistinction to the invasion of virulent pathogenic bacteria.

mice were found dead on the morning of the second day. One, on account of decomposition, was not examined; the other presented at the point of inoculation a slight reddish serous infiltration containing numerous streptococci. There was a moderate number in the spleen and blood from the heart. The two rabbits remained well. Cultures of this microbe were injected beneath the skin into the thorax and trachea of pigs without causing any disturbance."

Two series of inoculations in mice were made with this streptococcus for the purpose of testing the effect of repeated inoculations upon the virulence of the germ. The first mouse was inoculated with a pure culture, the second from the blood of the first and so on. The appended table is self-explanatory:

Inoculation experiments.

Mouse No.	Date of inoculation.	Inoculated subcutaneously with—	Date of death.	Time required to destroy life.	Remarks.
	1887.		1887.		
1	Aug. 15	2 to 3 drops bouillon culture.	Aug. 17	2 days	Streptococci in blood and spleen not found in cover-glass preparations from liver.
2	Aug. 17	Loop blood mouse 1..	Aug. 20	3 days	Do.
3	Aug. 20	Loop blood mouse 2..	Aug. 25	5 days	Do.
4	Aug. 25	Loop blood mouse 3..	Aug. 29	4 days	Do.
5	Aug. 29	Loop blood mouse 4..	.....	.....	Remained alive and well.

A second series gave the same result, showing that the streptococcus lost its virulence very rapidly.

Streptococcus B.—This streptococcus was obtained in pure cultures (bouillon and agar) from the spleen of a pig that died at the Experiment Station apparently from a disease of the liver. Mice inoculated subcutaneously with a pure culture of this microbe remained apparently perfectly well. A rabbit inoculated in a similar manner with 0.25 cc. of a turbid suspension in bouillon of the growth from an agar culture exhibited on the following day considerable reddening and slight swelling at the point of inoculation. No elevation of temperature. These symptoms soon disappeared.

Streptococcus E.—This streptococcus was obtained from the spleen of a pig. The animal was one among others that perished in an outbreak of modified hog cholera near Washington, D. C., in 1889. Streptococci C and D were obtained from pigs in the same outbreak.

Mice inoculated subcutaneously with from 1 to 3 drops of a bouillon culture died in from 2 to 14 days. The livers and spleens hyperæmic. Coagulation necrosis in the liver when the animals lived several days after inoculation. Stained cover-glass preparations from the organs showed the cocci single, in small clumps, and short chains, never in long chains. Pure cultures of the streptococcus were obtained from the organs in every case. A rabbit inoculated with one-eighth cc. of a bouillon culture in the ear vein died within 24 hours. Slight enlargement of spleen, liver hyperæmic, blood-stained serum in pericardial sac. In stained cover-glass preparations of the blood a few micrococci, usually in pairs, were discovered. No bacteria were observed in similar preparations from the spleen. Pure cultures of the streptococcus were obtained from both spleen and blood. A rabbit inoculated subcutaneously with one-fourth cc. of a bouillon culture was found dead on the seventh day. Surrounding the point of inoculation a slight injection of the blood vessels. Over the coils of the intestines, liver, and spleen a thin, semi-membranous exudate. Spleen somewhat enlarged, liver hyperæmic. The exudate contained a large number of single micrococci, also short chains. No bacteria were observed in stained cover-

glass preparations from the spleen, liver, and blood. A pure culture was obtained from the spleen. This streptococcus lost its virulence after having been cultivated for about one year, so that it would not destroy either mice or rabbits even when inoculated with very large quantities of a pure culture.

Streptococcus K.—This germ was associated with swine plague bacteria in a bouillon culture which was made from the peritoneal exudate of a pig that died of swine plague in an outbreak of that disease in New Jersey in 1890. (Streptococcus L was obtained from the liver of another pig in the same outbreak.) It produced no effect on mice. A rabbit was inoculated subcutaneously with 0.25 cc. of a turbid suspension of agar growth in bouillon. On the following day there was considerable reddening and slight swelling at the point of inoculation, temperature 104.4° F. (Normal temperature 103° F.) On the second day the rabbit appeared unwell; the fur was ruffled and it ate sparingly. Temperature 103.6° F. The next day it appeared well. Temperature normal and the local reaction had subsided.

Streptococcus Q.—This germ was found in large numbers in the lung of a cow affected with pneumonia (bronchial?). It produced no appreciable effect on mice. A rabbit inoculated in the ear vein with 0.25 cc. of a bouillon culture appeared unwell on the following day. Temperature 102.8° F. A rabbit inoculated subcutaneously in the ear with a loop of the surface growth from an agar-culture developed a small abscess at the point of inoculation, with slight reddening of the surrounding tissue. No other symptoms.

Streptococcus T.—This was obtained from a pleuro-pneumonia lung in 1891. Mice inoculated subcutaneously with a few drops of a bouillon culture died in from 3 to 4 days. No local reaction. Abdominal organs more or less hyperæmic. In cover-glass preparations from the spleen and liver were a large number of oval bacteria, surrounded apparently by a capsule. Pure cultures of the streptococcus were obtained from the blood. A rabbit inoculated in the ear vein with 0.50 cc. of a bouillon culture was found dead on the fifth day. The heart, muscles, and abdominal organs pale. About the right knee joint were several small abcesses in the inter-muscular tissue containing grayish pus. No other abcesses were found. No bacteria were observed in stained cover-glass preparations from the blood, liver, and spleen. In a similar preparation from the abcesses about the joint a large number of micrococci single, in pairs, and in short chains, were found. A pure culture was obtained from the blood.

Streptococcus U.—On June 24, 1891, two rabbits were inoculated with the mucus from the trachea of a horse which was killed on account of lameness. One of the rabbits died on June 28, from the organs of which this streptococcus was obtained. No other bacteria were discovered. Mice inoculated subcutaneously with a pure culture died on the fifth day. At the place of inoculation there was a purulent thickening of the skin and subcutis over an area one-half inch in diameter. Liver, spleen, and kidney swollen and discolored. No bacteria found in stained preparations made from the local lesions. Pure cultures were obtained from the spleen and blood.

A rabbit, which was injected subcutaneously with 0.10 cc. of a bouillon culture died on the second day. At the point of inoculation there was a slight injection of blood vessels. Liver pale, spleen enlarged, dark, and friable. Kidneys hyperæmic. Punctiform hemorrhages in lower colon. Lung emphysematous. Blood-stained serum in pericardial sac. Many cocci, usually in short chains, in cover-glass preparations from the organs. A pure culture from the blood. After four months this germ had lost its virulence to such a degree that a rabbit inoculated subcutaneously with 0.2 cc. of a bouillon culture remained well. It was reinoculated with 0.5 cc. five days later, and died on the second day following the inoculation. At point of inoculation a slight purulent infiltration into the subcutis, liver reddened, swollen, and sprinkled with minute round grayish areas (coagulation necrosis?). Spleen much enlarged, dark-colored, and friable. Considerable blood-stained liquid in

pericardial sac. Cover-glass preparations showed many micrococci, but no chains or capsules were observed. After five months (nine months in all) 0.75 cc. of a bouillon culture destroyed a rabbit in five days. A slight local reaction; exudative peritonitis; exudate contained cocci. Spleen dark. Liver enlarged, hyperæmic, kidneys reddened. Many bacteria appeared in preparations made from the liver, but few in those made from the blood. Pure cultures were obtained from the organs.

Streptococcus Z.—This germ was found in the liver of a pig that died in an outbreak of swine disease in Illinois in 1892. No hog-cholera or swine-plague bacteria were found in this animal, although the hog-cholera germ was found in several of the animals that perished in the same outbreak. Two mice inoculated subcutaneously with this streptococcus died, one on the fourth and the other on the fifth day. At point of inoculation a purulent thickening of the skin and subcutis extended over an area one-half inch in diameter. Livers pale (fatty), spleens and kidneys congested. Stained cover-glass preparations from the internal organs showed no bacteria, while those from local lesions exhibited a very large number of micrococci. Agar tubes inoculated with the blood of the first mouse that died, and with the spleen of the second one, developed pure cultures of the streptococcus. A rabbit inoculated subcutaneously with 0.75 cc. of a bouillon culture remained perfectly well.

Streptococcus α.—This organism was obtained from the blood of the same pig from which streptococcus Z was isolated. Mice inoculated with this germ died on the third day, and presented lesions similar to those produced by streptococcus Z. The local lesion was more extended in this case.

A rabbit that received 0.25 cc. of a bouillon culture in the ear vein was found dead on the following morning. Blood-stained liquid was found in the peritoneal cavity and pericardial sac. Heart muscle (right ventricle) hemorrhagic. Enormous numbers of oval bacteria, many of which exhibited a light central band, were found in cover-glass preparations from the various organs. They were usually united in short chains. A rabbit inoculated subcutaneously with 0.75 cc. of a bouillon culture remained well.

March 1, 1892, two pigs (Nos. 91 and 92) were each inoculated intravenously with 5 cc. of a bouillon culture. On the following day both animals were apparently unwell, ate sparingly, and moved about very little. March 4, improvement in appetite and general appearance, but both animals used the right fore leg very little. March 6, appetite fairly good, but pigs have no use of their right fore limbs. March 7, no apparent change in their condition when seen in the morning. At 1 p. m. pig 91 was found dead; examination made at once.

Pig No. 91 (female), Essex grade, aged 3 months, weight 65 pounds, condition good. The capsular ligament of the right shoulder joint thickened, deeply reddened areas on the inner surface, otherwise the capsule pale and apparently necrosed. Articular cartilage normal in appearance. An infiltration of a thick yellowish purulent substance in the intermuscular tissue surrounding the joint. Other joints normal.

In the abdominal cavity about 300 cc. of light amber-colored liquid, which coagulated on exposure to the atmosphere. A few elastic shreds adhered to the abdominal parietes. Spleen very much enlarged, discolored, and on the lesser surface several blood tumors. Liver swollen, hyperæmic. Kidneys swollen, surface mottled. A microscopical examination showed parenchymatous degeneration of both liver and kidneys. Stomach contained a small quantity of undigested food; mucosa of fundus slightly congested and pigmented. Upper portion of the duodenal mucosa sprinkled with stellate areas of blood injection; mucosa and contents deeply bile-stained. This was true of the small intestine throughout. Large intestine normal. Lungs hyperæmic and slightly emphysematous; a considerable area of the right lung agglutinated to costal surface by a fibro-gelatinous substance. An area 4 by 2 cm., of right lung, was deeply congested and partially hepatized. Heart well

filled with post-mortem clots; several punctiform hemorrhages on right auricle and base of right ventricle.

Stained cover-glass preparations from the various organs showed many micrococci, often in short chains. In the liver, the chains were from 4 to 8 cocci long, surrounded by an unstained border which appeared to be a capsule. From the peritoneal and pleural exudates, and from the organs pure cultures of the streptococcus were obtained.

Pig No. 95 remained apparently quite well, excepting the loss of the use of the right fore limb. Killed for examination March 23. Female, Essex, weight 90 pounds, in good condition. Right elbow joint considerably enlarged, articular surfaces normal, capsule very much thickened, cartilaginous and sprinkled with centers of ossification. Spleen considerably enlarged, engorged and on section shows a few hemorrhagic infarcts. Otherwise the tissues appeared to be normal. No bacteria found in cover-glass preparations made from the different organs, and media inoculated from the organs remained sterile.

The appended table contains a summary of the inoculation experiments with the pathogenic forms:

Summary of inoculation experiments with the pathogenic streptococci.

Streptococcus.	Fatal to—	Time required in destroying life.	Remarks.
A	Mice	2 days	Serous infiltration at point of inoculation; streptococci in spleen and blood.
B			Slight local reaction in rabbits.
E	Mice	2 to 14 days	Spleen and liver hyperæmic; coagulation necrosis in liver when life was prolonged for several days.
	Rabbits (ear vein)	24 hours	Spleen slightly enlarged.
	Rabbits (subcutaneously).	7 days	Local reaction; exudative peritonitis; many streptococci in exudate.
K			Local reactions and elevation of temperature in rabbits.
Q			Slight effect on rabbits; local abscess; no fever.
T	Mice	3 to 4 days	Spleen and liver hyperæmic.
	Rabbits (ear vein)	5 days	Small abscesses in intermuscular tissue.
U	Rabbits (subcutaneously).	2 days	Spleen and liver hyperæmic; punctiform hemorrhages in colon.
Z	Mice	2 to 4 days	Local reaction; spleen and liver hyperæmic.
	Mice	3 days	Local reaction; spleen and liver hyperæmic.
	Rabbit (ear vein)	24 hours	Spleen and liver hyperæmic; innumerable streptococci in organs.
α	Pig* (in vein)	7 days	Inflammation about right shoulder joint; spleen, liver, and kidneys hyperæmic; streptococci in organs.

*Streptococci A α and are the only ones that were inoculated into pigs.

Unfortunately streptococci Z and α perished before a further study could be made of their pathogenic properties.

Although the effect produced on animals by the nine pathogenic streptococci varied to a greater or less degree, in general the lesions produced were more characteristic of septicæmia and pyæmia than of any other general or local disease. The localization of the lesions in rabbits appeared only when the animals lived for several days—a condition comparable to that produced by attenuated rabbit-septicæmia bacteria. The comparatively rapid attenuation of the more virulent streptococci (excepting α, which perished before this point was determined) is also observable. This is important, as it points to a temporary virulence rather than to distinctive specific pathogenic species. The temporary virulence may, in some cases at least, be explained on the

ground suggested by Lesage and Macargne,* who discovered that *Bacillus coli comunis* acquired more or less virulence when exposed in diseased animal tissues.† Although we are not able to show that streptococci were in any case primarily the cause of the lesions in which they were found, or that they are capable of producing any disease in the experimental animals manifested by characteristic lesions,‡ their multiplication within the organs of the body undoubtedly had a more or less secondary influence in the production of the lesions from which they were obtained. The similarity that exists between the description of the streptococcus, supposed to be the cause of strangles and streptococcus U of this series, should be noted. The toxic effect of filtered or sterilized cultures of these streptococci was not tested.

The organs from which these streptococci were originally obtained presented such a variety of appearances that it was impossible to predict with any degree of certainty the presence of streptococci in any of the lesions examined. This is further evidence of their accidental invasion and non-specific etiological value. The results obtained and recorded in the preceding pages—limited and variable as they are—appear to be sufficiently clear to warrant the following conclusions:

CONCLUSIONS.

1. Streptococci are very generally distributed in nature, and especially on the mucous membranes of healthy animals. They fall very naturally into two classes: (1) Those that are strongly saprophytic in all of their properties; and (2) those that have decidedly parasitic tendencies in their morphological and cultural characters. The second class only has been found (by the writer) in diseased animal tissues.

2. Under certain conditions, presumably a weakened condition of the body with lesions of the mucosa in the air passages or intestinal tract, the streptococci of the mucous membranes invade the inner cavities and organs of the body. The large number of streptococci frequently found in the tissues indicate their invasion and subsequent multiplication prior to the death of the animal.

3. The streptococci that have been isolated in my investigations are capable of being differentiated by one or more distinct and apparently constant characteristics. In several instances, however, the differences are very slight, and may be due to previous conditions of life or unobserved irregularities in their cultivation.

4. Streptococci, quite as delicate and parasitic in their *cultural characters* as those obtained from diseased organs, are found in the flora of water and the mucosa of healthy animals, although no two of these

*Archiv. de Med., Exper. No. 2, 1892.

†A series of experiments is now in progress which it is hoped will throw some light upon this question with reference to certain streptococci.

‡Excepting more or less purulent infiltration of the subcutaneous tissue at the point of inoculation and general changes indicative of septicæmia or pyæmia.

contrasted species (?) have been found that possess precisely the same properties.

5. The grouping of the pathogenic and non-pathogenic streptococci by von Lingelsheim into *Streptococcus longus* and *Streptococcus brevis* does not hold true with all the forms that I have studied. The pathogenic effect of at least the majority of the destructive forms is septic in its character. Their virulence is soon lost.

6. Like other bacteria, streptococci are frequently found in the organs of animals that have perished from different and widely separated diseases. A few of these streptococci, as well as a small per cent of those from other sources, have been found to be fatal to certain of the experimental animals, while a large majority of them possess (when isolated) no pathogenic powers whatever as indicated by animal inoculations.

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A NON-MOTILE PATHOGENIC BACILLUS CLOSELY RESEMBLING THE BACILLUS OF HOG CHOLERA FOUND IN THE LUNG AND THE SPLEEN OF A PIG.

By VERANUS A. MOORE.

In the bacteriological examination of the spleen and a portion of the lung of a pig early in 1891, a non-motile bacillus was discovered which, on account of its pathogenic properties, has been somewhat carefully studied. Its appearance in a bouillon culture from the spleen and in stained cover-glass preparations from the bronchial secretions of the lung suggested a slightly modified form of the swine-plague germ. Further investigation, however, showed that biologically and in its effect upon animals it resembled very closely the hog-cholera bacillus. In the lung it was associated with attenuated swine-plague bacteria, but it appeared in a pure culture from the spleen. Unfortunately the other organs of this pig were not bacteriologically examined.

The pig from which this bacillus was isolated was of Essex grade, about 10 months old, and weighed 50 pounds. For several weeks prior to its death it had been kept in a pen at the Experiment Station with three other pigs. It had not been exposed to any swine disease. The other animals remained well. From the autopsy notes I take the following:

There was extensive broncho-pneumonia of the cephalic and median lobes, and small areas of hepatization in the principal lobes of both lungs. No pleurisy. Abdominal organs apparently normal. A comminuted fracture of the right femur and suppuration, the pus burrowing in all directions, but more especially downward. Both hock joints much enlarged and deformed. The injury to the femur was probably due to fighting.

The spleen and a small portion of the right lung* were removed with antiseptic precautions, and taken to the laboratory in a sterilized bottle and jar. These tissues were examined at once. No bacteria were found in cover-glass preparations from the spleen. A tube of bouillon inoculated with the spleen pulp developed a pure culture of a

*The piece of lung received at the laboratory was quite firm, pale, the lobules separated by opaque grayish lines, due to thickening of the interlobular tissue. The surface mottled with grayish, more or less elevated nodules, varying from one-half to two millimeters in diameter. On section the nodules appeared as grayish necrosed tissue. Upon pressure, muco-pus exuded from the larger bronchioles. Near the border there was ecchymosis beneath the pleura.

non-motile bacillus. Cover-glass preparations from the muco-pus from the bronchioles contained a very large number of oval, elongated bacteria slightly larger than the swine-plague germ. A smaller number of apparently the same bacteria were found in similar preparations from the consolidated lobules. A rabbit inoculated subcutaneously with the hepatized tissue died of swine plague on the fourth day. The characteristic polar-stained bacteria were found in cover-glass preparations from the organs. Plate cultures in agar developed colonies of swine-plague bacteria only. A second rabbit, inoculated with the muco-pus from the bronchioles, died on the eleventh day with lesions resembling those produced by hog-cholera bacteria. Pure cultures were obtained from the spleen, liver, and blood of a non-motile bacillus slightly larger than that of swine plague, but apparently identical with those obtained from the spleen of the pig.

The marked difference in the character of the lesions and the number of bacteria present in the organs of the two rabbits led to further investigations of the bacteria obtained (1) from the hepatized lung tissue, (2) from the muco-pus of the bronchioles, and (3) from the spleen. The methods employed and the results obtained were as follows:

INOCULATION OF RABBITS.

The necessary information concerning the inoculations for differential purposes is summarized in the appended table:

Rabbit No.	Date of inoculation.	Tissue and cultures inoculated subcutaneously.	Date of death.	Time required to destroy rabbits.	Remarks.
	1891.		1891.		
1	Jan. 9	Hepatized tissue pig's lung.	Jan. 14	5 days	Severe local reaction, peritonitis, swine-plague bacteria.
2	Jan. 24	0.01 cc. bouillon culture rabbit No. 1.	Jan. 28	4 days	Do.
3	Jan. 9	Muco-pus bronchioles, pig's lung.	Jan. 20	11 days ...	Local reaction; no peritonitis; necrosis in liver; very few bacteria in organs.
4	Jan. 22	0.01 cc. bouillon culture rabbit No. 3.	Jan. 26	4 days	Slight local reaction; no peritonitis; few bacteria.
5	Jan. 17	0.01 cc. bouillon culture pig's spleen.	Jan. 20	3 days	Slight local reaction; necrosis in liver; few bacteria.

The examination of rabbits Nos. 1 and 2 showed typical lesions of attenuated swine plague. There was severe local reaction and peritonitis with the polar-stained bacteria in cover-glass preparations. The lesions in rabbits Nos. 3, 4, and 5 were characterized by local reaction, which was slight in Nos. 4 and 5; enlarged, discolored spleen; necrosis in the liver; heart muscle pale. In stained cover-glass preparations from the spleen and liver were very few elongated oval bacteria slightly larger than the swine-plague germ and usually united in pairs. They exhibited *a light unstained center and deeply stained periphery.* In similar preparations from the blood a much smaller number of bacteria was found.

The fact that the rabbit which was inoculated with the virus from the bronchioles lived a day longer than the one inoculated with the culture from the spleen, is worthy of note, as it points to the supposition that the bacteria after penetrating the spleen had become slightly increased in their virulence.

It should be observed that in the cover-glass preparations from the bronchial secretions and the hepatized lung tissue of the pig there was not the visible difference in the size of these bacteria that was apparent in the preparations made from the organs of rabbits that perished from their inoculation, or from subsequent cultures.*

CULTURE TEST.

Repeated cultures were made on all of the ordinary media of the bacteria obtained from the organs of the pig. Those made from the hepatized lung tissue and from the germ obtained from the rabbit inoculated with the same could not be distinguished from cultures of swine-plague bacteria. The cultures made from the bacteria obtained from the spleen and from the muco-pus of the bronchioles, by rabbit inoculation, could not be differentiated. They were identical in every respect, and resembled similar cultures of hog-cholera bacteria as the subsequent description will show.

DESCRIPTION OF THE BACILLUS.

Morphology.—A non-motile, rod-shaped germ, from 1.0 to 2.0μ in length and from 0.7 to 1.2μ in thickness. In stained cover-glass preparations from an agar culture they are about 1.0μ long and from 0.7 to 0.8μ wide; the ends are rounded, giving them the appearance of elongated ovals. In bouillon they are appreciably larger, usually single, but occasionally united in pairs. In stained preparations from the tissues of rabbits they are much larger (1.5 to 2.0μ long and from 0.8 to 1.2μ wide). The periphery stains more deeply than the central portion; this is also frequently observed in preparations from cultures. It does not retain its coloring matter when treated after the Gram or Weigert-Gram methods.

Biology.—Agar at 36° C. On the surface of agar, after 24 hours,

* The non-appearance of the swine-plague germ in the organs of the rabbit inoculated with the muco-pus of the bronchioles is explained by the fact that they were very largely localized in the hepatized tissue as shown from the plate cultures. In preparing the material for inoculation the surface of the lung was scorched and an incision made with a sterile knife. The section was then scorched and the muco-pus obtained by pressure. The few swine-plague bacteria that were presumably present in the secretions were, on account of their attenuation, overcome by the great excess of other bacteria. It is probable that the swine-plague germs in this case belonged to the tracheal bacteria not distinguishable from those of swine plague that are normally present in the air passages of many healthy swine. (See report on Swine Plague, U. S. Department of Agriculture, 1891, p. 107.)

round, convex, grayish, non-viscid colonies appear, varying from 0.5 to 1.5 mm. in diameter, according to the number present. The edge of the colonies is sharply defined, the surface glistening. Within the agar small grayish colonies develop. When grown on agar plates ("double dishes") a peculiar, somewhat musty, penetrating odor is given off.

Gelatine.—At the ordinary temperature the growth is quite slow. After several days round, convex, glistening colonies are developed. Within the gelatine the colonies are smaller, 0.7mm. in diameter, which have, under a hand lens, a slightly yellowish color and homogeneous appearance. The gelatine is not liquefied.

Potato.—At 36° C. a thin, brownish growth is formed over the surface. The growth continues slowly for several days. In old cultures it is of a yellowish brown color.

Alkaline peptonized bouillon.—After twenty-four hours, at 36° C., the liquid is uniformly heavily clouded with a very slight quantity of a grayish sediment. No appreciable odor. *Acid* reaction, a few bubbles at surface. In two weeks the liquid is nearly cleared, with the formation of a grayish, friable sediment. At this time the reaction is alkaline.

Acid peptonized bouillon.—In this medium the growth is quite feeble, imparting a faintly uniform cloudiness to the liquid. A grayish band composed of bacteria is formed on the sides of the tube at the surface of the liquid. The reaction becomes alkaline in about two weeks.

Alkaline peptonized bouillon, plus 2 per cent glucose.—In fermentation tubes containing this liquid the growth is quite vigorous throughout. The liquid becomes clouded and acid after twenty-four hours. Gas is formed, which replaces about one-third of the liquid in the closed bulb. The fermentation is very slow; only a few bubbles of gas appear in the first twenty-four hours. The fermentation is completed in about six days. When treated with a solution of caustic potash about one-third of the gas is absorbed. When the remaining gas is brought in contact with a flame there is a feeble explosion.

Milk.—In this medium, at 36° C., the growth is vigorous. In stained cover-glass preparations, the bacteria are often observed in clumps. The milk remains unchanged in appearance for about two weeks, when it presents a pearly, semi-translucent appearance. A microscopical examination shows that the fat globules are destroyed.* It is strongly alkaline in reaction. The casein is precipitated by the addition of a few drops of acetic acid. There is a doubtful peptone reaction.† A few experiments were made to test its power of resisting drying, and to determine its thermal death point.

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*This peculiar appearance of the milk is also produced by the hog-cholera bacteria, but usually a much longer time is required.

†As a few drops of caustic potash or soda solution added to a tube of milk will produce the same appearance, it is probable that the change is largely due to the alkali which is produced during the multiplication of the bacteria.

Drying.—Cover-glasses were placed on a glass stand and covered with a bell jar, all of which were previously sterilized. A drop of a bouillon culture, one day old, was placed, by means of a flamed pipette, on each cover-glass. A tube of bouillon was inoculated with one of these cover-glasses on each day for seven consecutive days. The tubes inoculated on the fifth, sixth, and seventh days remained sterile. The others developed pure cultures of the bacillus.

Thermal death point.—Five tubes of bouillon were inoculated with about six drops each of a fresh bouillon culture and heated in a water bath for five, ten, fifteen, twenty, and twenty-five minutes at 58° C. The tube that was heated for twenty-five minutes remained clear. All of the others, including check, developed pure cultures of this bacillus.

PATHOGENIC PROPERTIES.

In the detailed account of the isolation and differentiation of this bacillus, its effect upon rabbits was partially described. The germ (the one from the spleen) was preserved by means of subcultures until September (eight months), when a series of inoculations were made which demonstrated more fully the range of its pathogenic effect. Rabbits inoculated subcutaneously with 0.01 cc. of a bouillon culture died in from four to six days. The lesions were marked necrosis in the liver, enlarged and discolored spleen, heart muscle pale. In some cases Peyer's patches were pigmented and the kidneys more or less hyperæmic. Occasionally a few shreds of exudate over the intestines, but no bacteria present as indicated by cover-glass preparations. The organs contained very few elongated oval bacteria which exhibited a light unstained center and deeply stained periphery in cover-glass preparations. This was true in similar preparations from other animals. An intravenous inoculation of the same quantity proved fatal in about forty-eight hours. Guinea pigs were destroyed in eight days by a subcutaneous injection of 0.03 cc. of a bouillon culture. The lesions were similar to those produced by hog cholera bacteria. Mice perished in six days after a subcutaneous injection of from one to two drops of a bouillon culture.

A pig weighing 50 pounds, inoculated intravenously with 6 cc. of a four-day bouillon culture, died on the afternoon of the second day. The lesions were not distinguishable from those produced by a similar inoculation of strong hog cholera virus. Pure cultures were obtained from the different organs.

A rabbit that had been made immune to swine plague was inoculated subcutaneously, together with a check rabbit, with 0.01 cc. of a bouillon culture. The check died in four days. The treated rabbit perished on the sixth day, two days later than the check. This is interesting, as rabbits that have been made immune to swine plague offer no resistance whatever to hog-cholera bacteria.

In June, 1892, this bacillus was as fatal to rabbits as in September,

1891. This is important in differentiating it from certain so-called toxicogenic bacteria that have been found in water and in diseased animal tissues which were fatal to rabbits when first isolated, but which soon lost their virulence.

From the description of the various properties of this bacillus it will be observed that it resembles the hog-cholera bacillus in (1) its biological characters; (2) the light center and deeply stained periphery in cover-glass preparations;* (3) the appearance of the bacteria in pairs in preparation from animal tissues, and (4) the character of the lesions produced in experimental animals. It differs from it in (1) morphology, (2) its feeble resistance to drying, (3) the small number of bacteria present in the tissues of animals dead from its inoculation, and (4) it is more rapidly fatal to rabbits. Its non-motility, however, is the only *specific* difference that can be considered between it and the bacillus of hog cholera. Cultures on the various media have been very carefully examined in the very early and advanced stages for the purpose of determining whether or not this germ was motile under any of the ordinary conditions of cultivation, or at any age of the culture, but only negative results were obtained. No flagella could be found, although a very careful examination was made in which the same methods were employed that were satisfactory in demonstrating the flagella on the hog-cholera, coli, and typhoid bacilli. As flagella can be demonstrated on motile but not on non-motile bacteria, it seems fair to presume that after these tests this bacillus can be safely considered a non-motile organism.

Although morphologically it resembles the swine-plague group of bacteria its cultural characters and pathogenic effect on animals differentiate it very distinctly from that group of organisms. The differences between it and the swine-plague and hog-cholera bacteria appear to be sufficiently great to consider it a species rather than a modified form of either of the specific disease germs. Further investigations, however, may reveal intermediate forms which will connect it unquestionably with one or the other of these specific bacteria. The close resemblance that exists between these organisms suggests the importance of a thorough examination of all of the various properties of a germ before it is placed unreservedly in any species, and especially so in cases of pathogenic organisms where a description based on incomplete observations may lead to unexplainable differences in the correlation of our disease-producing bacteria.

* In the earlier investigations of hog cholera (Second Annual Report Bureau of Animal Industry, U. S. Department of Agriculture, 1885, p. 212) this reaction of the bacteria to the staining fluids was very constant. It is not, however, so universally observed in the bacteria that have been isolated from more recent outbreaks, although it is frequently noticed. The difference may be due to slight variations in the germs themselves, or to some inappreciable difference in the staining fluids or mode of their application.

From a bacteriological standpoint the natural habitat of this bacillus is of much interest. The fact that it has not heretofore been observed would indicate that it does not belong to the normal flora of the lungs. As it was the predominating form in the bronchial secretions, it is presumable that it first gained entrance to the air passages, from which it invaded the inner organs of the body. Although it was universally fatal to experimental animals, there is very little if any evidence that it was to any degree responsible for the death of the pig from which it was obtained. This fact renders it of little economic importance when considered with reference to this case alone, but it seems reasonable to suppose that a germ which possesses such marked pathogenic properties might, under certain conditions, be the cause of a more or less serious outbreak of infectious disease.

PATHOGENIC AND TOXICOGENIC BACTERIA IN THE UPPER AIR PASSAGES OF DOMESTICATED ANIMALS.

By VERANUS A. MOORE.

In 1888 Dr. Theobald Smith* demonstrated the fact that in the secretions covering the posterior nares, pharynx and larynx of certain numbers of apparently healthy pigs, were bacteria, which could not be distinguished either by their cultural characters or in their pathogenic properties from the swine-plague germs. In an appendix to the same report are the results of a few preliminary examinations of the secretions from the upper air passages of certain other domesticated animals which I made for the purpose of determining the extent of the distribution of these bacteria. In these investigations it was found that similar bacteria were present in the secretions covering the mucosa of the upper air passages in certain numbers of such animals as cattle, dogs, and cats. Since the publication of that report, further examinations have been made, from time to time, from both the same and other species of animals. As a final result of these observations the swine-plague group of bacteria was found to be present, apparently in its natural habitat, in the upper air passages of a certain per cent of healthy pigs, cattle, cats, dogs, and horses. In sheep very attenuated forms were occasionally found. Pathogenic bacteria were not discovered in the flora of the mucosa of healthy rabbits, fowls, and guinea pigs. A few examinations were also made from frogs and turtles, but with negative results.

The method that was employed in the latter examinations is the same as that used in the earlier investigations, viz, the subcutaneous inoculation of rabbits with the secretions taken from the mucosa of the upper air passages of the living or freshly killed animals. It is given in detail in the report on swine plague (*l. c.*, p. 110), and consequently need not be repeated here.

The pathogenic bacteria previously reported from the healthy mucosa of the different species of animals could not be distinguished from the swine-plague group, and only slight variations were found to exist between the germs isolated from the various animals, either of the same

*Report on the Cause and Prevention of Swine Plague. U. S. Department of Agriculture, 1891, p. 109.

or different species. The more recent investigations, however, have revealed the existence of bacteria which vary in a marked degree from the more typical swine-plague germ, and also the presence of toxicogenic bacteria which are widely separated from the swine-plague group of organisms.

The total results, as reported in 1891, are, for the sake of comparison, tabulated here. As previously stated, all the pathogenic bacteria which were obtained could not be distinguished from the swine-plague germs. They varied from the rapidly fatal septicæmia forms which destroyed rabbits in sixteen hours when inoculated subcutaneously, to those that possessed so little virulence that thirteen days were required for the rabbits to die.

Table showing the species of animals from which rabbits had been inoculated and the results reported up to 1891.

Species of animals.	Number of animals from which rabbits were inoculated.	Swine-plague bacteria present in.	Number pathogenic bacteria not found in.	Per cent normally infected.	Virulence of the germs.
Pigs	19	5	14	26	Attenuated.
Cattle	7	5	2	71	Do.
Cats	7	6	1	85	Very virulent and attenuated.
Dogs	6	2	4	33	Virulent.
Horse	1	.....	1	.....	No pathogenic bacteria.
Sheep	1	.....	1	.....	Do.
Fowls	2	.....	2	.....	Do.
Rabbit	1	.....	1	.....	Do.

In addition to the inoculations from pigs which were reported, another series was made which gave uniformly positive results. The bacteria, however, as in the other cases were attenuated, producing in the rabbit more or less cellular infiltration with the localization of the bacteria on one or more of the serous membranes. The facts necessary for an understanding of these inoculations are given in the appended table:

Inoculation of rabbits with the mucus from the upper air passages of pigs.

Pig No.	Mucus from—	Rabbit inoculated.	Date of inoculation.	Rabbit died in—	Remarks.
		No.		*Days.*	
181	Larynx	1	Sept. 23, 1889	2	Local reaction; peritonitis.
		2	....do	2	Do.
267	Trachea	3	Jan. 2, 1890	5	Do.
		4	....do	6	Local reaction; hemorrhages in cæcum.
303	Pharynx	5	Jan. 28, 1890	3	Local reaction; peritonitis.
		6	....do	4	Do.
305	Larynx	7	....do	4	Do.
		8	....do	5	Local reaction; hemorrhagic areas in cæcum.
308	Pharynx	9	Feb. 11, 1890	4	Local reaction; peritonitis; pleuritis.
		10	....do	5	Do.
219	....do	11	Feb. 14, 1890	3	Do.
		12	....do	.....	Remained apparently well.
202	Larynx	13	....do	4	Local reaction; peritonitis; hemorrhages in cæcum.
		14	....do	4	Local reaction; pleuritis.
7	....do	15	Apr. 2, 1890	4	Local reaction; peritonitis; hemorrhages in cæcum.

The pigs from which these inoculations were made came from different herds, excepting Nos. 181 and 305. These were secured from the same farm with an interval of nearly nine months between the times of purchase. The inoculations were made in a few cases as soon as the pigs were purchased, but in other instances they were kept at the Experiment Station for a greater or less period of time prior to the examination. The uniform positive results obtained in this series would indicate a more general distribution of the swine-plague group of bacteria in the flora of the upper air passages of healthy pigs than would be supposed from the previously reported results.

In the consideration of the more recent rabbit inoculations from the various animals, a table has been constructed for each of the species examined. Very few additional examinations have been made from those species from which a considerable number of examinations were heretofore reported. It seemed, for obvious reasons, more desirable to obtain the secretions from freshly killed animals, and on this account the work has extended over a considerable length of time in order to obtain the secretions from suitable animals that were killed for other and more important purposes. In a few instances the cattle and sheep were slaughtered at the abattoir for beef and mutton. I am indebted to Dr. F. L. Kilborne, Director of the Experiment Station, for assistance in making many of the inoculations.

INOCULATIONS FROM CATTLE.

Rabbits have been inoculated with the secretions from the upper air passages of three animals. Two of these (Nos. 8 and 9), were from the Experiment Station, and the other (No. 10) was a steer killed at the abattoir for beef. No. 8 died of Texas fever. The mucus was taken from the amygdaloid cavities very soon after death. This fact does not detract from the justice in using this animal, as the disease from which she suffered was not bacterial in its nature. The subjoined table is self-explanatory:

Inoculation of rabbits with the mucus from the upper air passages of cattle.

Animal No.	Mucus from—	Rabbit inoculated.	Date of inoculation.	Rabbit died in—	Remarks.
		No.		*Days.*	
8	Amygdaloid cavities..	27	Aug. 27, 1891	2	Local reaction, spleen, liver, and kidneys hyperæmic.
	.....do	28	....do	2	Local reaction, peritonitis.
9	.....do	29	May 13, 1892	15	Severe local reaction, spleen and liver hyperæmic.
10	Larynx	30	June 2, 1892	5	Severe local reaction, thoracic and abdominal organs normal, cultures remained clear.

The bacteria* obtained from rabbits inoculated from cow No. 8 could

*The various morphological, biological, and pathogenic properties of these bacteria will not be mentioned excepting where they differ from the descriptions published in the previous report (*l. c.*) or where additional facts concerning any of these properties have been obtained.

not be distinguished from the swine-plague germ in their morphology, biological characters, or pathogenic properties. They gave the phenol reaction, but no trace of indol could be found. They perished after drying on cover-glasses for twenty-four hours, and were destroyed by heating at a temperature of 60° C. in a water bath for ten minutes.

In order to compare more fully the pathogenic effect of these bacteria with that of the swine-plague germ a pig was inoculated with a culture from rabbit No. 27. The facts concerning the inoculation and its results are as follows:

October 16, 1891.—Pig No. 39, weighing about 25 pounds, was inoculated with 5cc. of a fresh bouillon culture in a subcutaneous vein in the right thigh.

October 17.—Pig barely able to stand.

October 19.—Pig down, unable to rise; ate very little.

October 20.—Pig lies on one side, can not be roused up; refuses food.

October 26.—The pig still refuses food. Metatarsal joints considerably swollen. It breathes with apparent difficulty.

October 27.—No appreciable difference in its condition. It was killed by a blow on the head.

Post-mortem examination.—The skin over the ears and above the eyes and nose was of a purplish color; a small amount of subcutaneous fat. Both metatarsal joints and right elbow joint swollen, fluctuation detected. Subcutis about these joints œdematous. The sections showed the joint capsules to contain a considerable quantity of thick, greenish-yellow, semi-solid substance. About the edges of the joint cartilages were small pockets of pus. The suppuration extended from the capsule between the muscles for from 1 to 2 inches. Spleen slightly enlarged, pulp firm. Liver slightly discolored, fatty, contained several cysts of *Tænia echinococci.** The gall bladder contained a small quantity of very thick, yellowish-brown bile which could not be forced through the duct. Kidneys, cortex pale (parenchymatous degeneration), pyramids of a glairy, reddish appearance. Capsule easily removed. Stomach contained no food; a small quantity of bile-stained mucus in pyloric end. Mucosa pale. Small intestines contained bile-stained mucus; mucosa slightly reddened from injected blood vessels. Lymphatics pale and œdematous throughout. Lungs partially collapsed, deeply congested, but no hepatization or hemorrhages. Heart muscle very pale.

Stained cover-glass preparations from the blood, spleen, liver, and pus from elbow joint showed no bacteria. Similar preparations from the kidneys exhibited a large number of oval bacteria, many of which took the polar stain. Tubes of agar and bouillon inoculated from the blood and spleen remained clear. Those inoculated from the liver, kidneys, and pus from the affected joints developed pure cultures of bacteria which were not distinguishable from the swine-plague germ.

A rabbit was inoculated subcutaneously with a bit of the pus from one of the metatarsal joints. It perished on the third day with lesions characteristic of attenuated swine plague.

The suppurative condition of the joints which formed the most marked lesion is not unlike that found by Dr. Smith in pigs inoculated intraveneously with swine-plague bacteria (*l. c.* p. 75). The resemblance of this germ to that of bovine pneumonia is worthy of careful attention.

In the rabbit inoculated from cow No. 9 there was a marked difference. It lived for an unusually long period after the inoculation. The

*This appears to be the first time that this parasite has been found in pigs from the District of Columbia.

subcutaneous and intermuscular tissue was infiltrated with a purulent substance over the entire abdomen and thorax. The abdominal organs were hyperæmic but no exudate. Cover-glass preparations revealed the presence of no bacteria, but culture media inoculated from the spleen and blood developed feeble growths of an elongated non-motile bacillus, which will be briefly described under the provisional name of bacillus No. 1.

BACILLUS No. 1.—*Source:* From the spleen and blood of a rabbit inoculated with the mucus from the amygdaloid cavities of cow No. 9. *Morphology:* A non-motile oval bacillus 1.5 to 2.0μ in length. Stains deeply or with a light center. Polar arrangement of the cellular protoplasm well marked when examined at the edge of a hanging drop preparation. *Biology:* On the surface of agar it develops a somewhat vigorous grayish, non-viscid growth. Growth very feeble in gelatine. It does not develop on potato. Bouillon becomes very faintly clouded with an acid reaction. In peptonized bouillon containing 2 per cent glucose in a fermentation tube it imparts a uniform cloudiness to the liquid throughout the tube. It ferments the sugar with the production of a large quantity of gas (five-ninths of the contents of the tube) one-third of which was absorbed by potassium hydrate. Milk was thickened in twenty-four hours, not viscid. *Pathogenesis:* Rabbits inoculated with 0.5 cc. of a fresh bouillon culture exhibited an elevation of temperature which lasted for about five days. Otherwise they appeared to be unaffected.

Rabbit No. 30, inoculated from cow No. 10, exhibited an extensive purulent infiltration and sanguinolent effusion in the subcutaneous tissue over the entire ventral aspect of the body. No bacteria were found in the organs, but cover-glass preparations from the local lesion contained a very large number of oval elongated bacteria, resembling those isolated from rabbit No. 29. Unfortunately no cultures were made from the local lesion and those made from the spleen and blood remained clear.

INOCULATIONS FROM SHEEP.

The sheep from which secretions were obtained were killed in part for mutton at the abattoir and in part for various purposes at the Experiment Station. In the examinations that have thus far been made no bacteria have been found which were identical in their various properties with the swine-plague group of organisms. The facts necessary for an understanding of these inoculations are tabulated below.

Inoculation of rabbits with the mucus from the upper air passages of sheep.

Sheep No.	Mucus from—	Rabbit inoculated.	Date of inoculation.	Rabbit died in—	Remarks.
		No.		*Days.*	
2	Larynx and pharynx.	33	Nov. 6, 1891	10	Severe local reaction; bacteria from local lesions only.
	....do	34	.....	.....	Killed after thirty-two days; no bacteria.
3	Mouth	35	May 26, 1892	18	Local abscess; oval bacteria from local lesion only.
4	....do	36	....do	19	Severe local reaction; no pathogenic bacteria.
5	....do	27	....do	.....	Rabbit remained well.
6	Pharynx and larynx.	38	May 28, 1892	22	Slight local reaction; many coccidia in liver, no bacteria.
	....do	39	....do	.....	Rabbit remained well.

Rabbit No. 33 exhibited an extensive purulent infiltration in the subcutaneous tissue over the ventral aspect of the body, starting from the point of inoculation. The thoracic and abdominal organs were apparently normal. No bacteria were found in cover-glass preparations made from the spleen and blood. A large number of oval, elongated bacteria were present in similar preparations from the local abscess. Cultures from the spleen and blood remained clear. A tube of bouillon inoculated from the local abscess developed an impure culture of a bacillus resembling morphologically the swine-plague germ. The other rabbit was killed after thirty-two days. No lesions.

A rabbit was inoculated subcutaneously with 0.50 cc. of a bouillon culture of the germ made directly from the local abscess of rabbit No. 33. It perished on the second day. It exhibited slight local reaction; liver large, hyperæmic. Spleen slightly enlarged. No localization of bacteria on serous membranes. Bacteria appeared in cover-glass preparations from the liver as deeply stained bodies. No polar stain observed. Similar preparations from the spleen and blood exhibited no bacteria. Tubes of bouillon inoculated with bits of the liver developed pure cultures of a non-motile bacillus 1.5 to 2.5 μ in length; ends rounded. At the edge of a hanging drop preparation the cell protoplasm was observed to be more dense at the poles or extremities. Many of them exhibited the polar stain. The periphery stained more deeply than the center. The cultures from the spleen and blood remained sterile. This bacillus differed very slightly from the swine-plague germ in its biological properties.

The rabbit inoculated from sheep No. 4 presented on post-mortem examination an extensive purulent infiltration into the subcutis. No inflammation of the serous membranes. From the local lesion cultures of an elongated, oval germ were obtained which subsequently destroyed a rabbit in three days when inoculated subcutaneously with 0.75 cc. of a pure bouillon culture. It exhibited a local reaction, but no localization of the bacteria on the serous membranes. This germ differed from the swine-plague organism in (1) not exhibiting a polar stain in cover-glass preparations from the organs of the rabbit; (2) it was slightly larger; (3) the growth on the agar was not viscid, and (4) its localization at the place of inoculation only.

Rabbit No. 36 perished evidently from the effect of the local reaction, which was extremely severe. A micrococcus which grew in clumps was isolated from the infiltrated substance, but it possessed no pathogenic properties, as indicated by an intravenous inoculation in rabbits. Rabbit No. 38 perished presumably from the effect of the coccidia in the liver.

Although the bacteria isolated from the air passages of sheep differed in a marked degree from each other, and from the swine-plague germs in their virulence and pathogenic manifestations, their general morphological and biological characters would indicate that they

belong to that group of organisms. The severe purulent infiltration into the subcutaneoue tissue, extending from the point of inoculation, was undoubtedly partially due to other germs that were introduced with the mucus. The non-localization of the bacteria on the serous membranes is the most important variation that was found to exist between these and the attenuated swine-plague bacteria.

INOCULATIONS FROM HORSES.

Rabbits were inoculated with the secretions from the upper air passages of five horses. Two of these were the work horses at the Experiment Station. The other three were from Washington, D. C., two of which were killed for lameness. The following table is self-explanatory:

Inoculation of rabbits with the mucus from the upper air passages of horses.

Horse No.	Mucus from—	Rabbit inoculated.	Date of inoculation.	Rabbit died in—	Remarks.
		No.		*Days.*	
2	Trachea	42	June 24, 1891	5	Spleen enlarged; streptococci from organs.
	Larynx	43	....do	9	Slight local reaction; peritonitis; polar-stained bacteria.
3	Root of tongue	44	May 26, 1892	.....	Rabbit remained well.
4	Nares	45	Apr. 23, 1892	.....	Do.
		46	....do	.....	Do.
5	Pharynx	47	Mar. 8, 1892	.....	Do.
6	Root of tongue	48	Apr. 7, 1892	26	Rabbit emaciated; no bacteria.
		49	....do	.....	Rabbit remained well.
		51	....do	.....	Do.

The inoculations with the mucus from horses showed the flora of their mouth, nose, and throat to be comparatively free from pathogenic bacteria. No. 2, however, was unusually fertile in these forms. Rabbit No. 42 died evidently from the effect of a streptococcus which was isolated from its organs. This germ was rapidly fatal to rabbits and mice; 0.10 cc. of a boullion culture destroyed a rabbit, when inoculated subcutaneously, in less than two days. In cover-glass preparations from the organs of the rabbit it appeared very largely in single forms, frequently, however, in very short chains. The individual segments were elongated and frequently exhibited a polar stain. The resemblance of the individual elements to the swine-plague germ was very marked. This was true of its biological properties. It became much attenuated in about nine months. This germ is described more fully under the name "Streptococcus U," in the article on Streptococci.

Rabbit No. 28 presented lesions typical of attenuated swine plague. Its organs contained bacteria that could not be differentiated from the swine-plague germ.

A rabbit inoculated subcutaneously with 0.10 cc. of a bouillon culture died on the third day with severe local reaction, but no marked localization of the bacteria on the serous membranes. No phenol or indol reaction could be detected in bouillon cultures of this germ. Its power

to resist drying and its thermal death point were not appreciably different from those of the swine-plague germ.

Rabbit No. 33 perished presumably from the effects of the inoculation, but no bacteria could be found in any of the organs. The other rabbits remained well.

INOCULATION OF GUINEA-PIGS FROM THE NASAL DISCHARGE OF A MULE.

September 10, 1892, Dr. F. L. Kilborne inoculated a guinea-pig with mucus from the nose of a mule which was suspected of having glanders. The guinea-pig was found dead September 12. From the organs cultures of bacteria were obtained which were fatal to rabbits in from two to four days. They were not distinguishable from the swine-plague germ in their biological and pathogenic properties. In cover-glass preparations from tissues, however, they did not exhibit the polar stain.

September 12 a second guinea-pig was inoculated from the same mule. It died September 14. Tubes of bouillon inoculated from the organs developed pure cultures of the *Bacillus fluorescens liquefaciens*. A rabbit inoculated subcutaneously with 0.75 cc. of a bouillon culture of this bacillus perished in forty hours. At the point of inoculation there was an infiltration of serum into the subcutaneous tissue. Abdominal organs hyperæmic. Cover-glass preparations from the spleen and liver contained many bacilli. Pure cultures were obtained from the spleen and liver. A rabbit inoculated in the ear vein with 0.10 cc. of a fresh bouillon culture perished in seven days. After three weeks cultivation this germ lost its virulence to such an extent that 0.75 cc. produced no effect on rabbits. This is the first time, so far as I am aware, that this bacillus has been reported as possessing pathogenic or septic properties.* It did not produce gas. When grown in the fermentation tube it developed in the open bulb only.

INOCULATIONS FROM CATS.

Rabbits have been inoculated with the mucus from the upper air passages of three healthy cats, all of which contained bacteria which belong to the swine-plague group of organisms. The facts concerning these inoculations are tabulated below.

*In the summer of 1892, while making a series of inoculations in pigeons with another germ, the cultures became contaminated with the *Bacillus fluorescens liquefaciens*. It was isolated by means of agar plate cultures and a pigeon inoculated in the pectoral muscle with 0.50 cc. of a pure bouillon culture. The pigeon died in forty hours with extensive necrosis of the pectoral muscle. Heart muscle pale. Many bacilli were found in cover-glass preparations from the organs. A second pigeon, inoculated with 0.50 cc. of an emulsion of the pectoral muscle from the first pigeon, remained well.

Inoculation of rabbits with mucus from the upper air passages of cats.

Cat No.	Mucus from—	Rabbit inoculated.	Date of inoculation.	Rabbit died in—	Remarks.
		No.		*Hours.*	
8	Larynx.....	63	Aug. 31, 1890	21	Septicæmia.
		64	Aug. 31, 1890	21	Do.
9	Trachea and larynx.	65	Sept. 4, 1892	20	Do.
10	Pharynx and larnyx.	66	May 31, 1892	48	Local reaction; peritonitis.

The bacteria obtained from cat No. 8 by rabbit inoculation was compared very carefully with the germs of acute swine plague. They gave in bouillon (containing 1 per cent peptone) cultures 22 days old a decided phenol reaction, but no trace of indol could be found. Their power to resist drying and their thermal death point were identical with those of the swine-plague germ.

The pathogenic power of these bacteria was tested by a pig, guinea-pig, and rabbit inoculations.

October 16, 1891.—A pig weighing about 30 pounds was inoculated in the vein (inside of right thigh) with 5 cc. of a fresh bouillon culture. It was found dead on the morning of October 17, twenty-four hours after its inoculation. The abdominal organs were slightly hyperæmic, lymphatic glands reddened. About 100 cc. of a clear straw-colored serum in the right pleural cavity, a smaller quantity on the left side. Beneath the pleura over the lungs were several irregular areas containing petechiæ. Lungs not collapsed. Slight emphysema. Cover-glass preparations from the organs contained very many polar-stained, oval bacteria.

A pig inoculated at the same time, and with a similar quantity of a like culture of virulent swine-plague bacteria, perished at the same time and with similar lesions.

A guinea-pig inoculated subcutaneously with 0.001 cc. of a bouillon culture perished on the tenth day. It exhibited considerable cellular infiltration into the subcutis, involving the superficial layer of the subjacent muscle. The abdominal organs were covered with a cellular, pasty exudate. There was beginning pleuritis as exhibited by a large number of bacteria in cover-glass preparations made by drawing a cover-glass over the surface of the visceral pleura. A guinea-pig inoculated with a similar quantity of a like culture of a virulent swine-plague germ recovered after some weeks. This fact would indicate a slight increase in the virulence of the tracheal germs over that of the swine-plague organism used.

They destroyed rabbits in about sixteen hours. In cover-glass preparations from their organs the bacteria were appreciably larger than the swine-plague germ. They appeared frequently in clumps, and when stained were separated by unstained septa, which suggested the possibility of a capsule. There were many involution forms, in some of which the cellular protoplasm was condensed near the center of the cells rather than at the ends as indicated by their reaction to the staining fluids.

The bacteria from cat No. 9 differed from those from cat No. 8, in that they were larger, did not exhibit the polar stain (excepting involution forms), and were localized more especially in the liver of the rabbit.

Rabbit No. 66 exhibited lesions characteristic of attenuated swine plague.

The almost uniform presence of the swine plague group of bacteria in the normal saliva of cats is of considerable interest, inasmuch as these animals are so largely the living pets of children and the effect of these germs on the human species is not yet demonstrated. Their effect on the smaller experimental animals, however, does not differ, to any marked degree, from those of the germs of pneumonia in man.

Fiocca* has recently described a pathogenic bacillus which he found in the saliva of cats and dogs, and which resembles the germ of rabbit septicæmia, but only one-half its size. The variation which I have found in the size of the bacteria from the saliva of cats would indicate that Fiocca's germ belongs to the same group of bacteria as those which I have found in these animals. It will be observed that there is a considerable variation in both the morphology and virulence of the pathogenic bacteria found in the upper air passages of apparently healthy cats.

OTHER INOCULATIONS.

Rabbits were inoculated with the secretions from the pharynx, larynx, or trachea of one healthy dog, six rabbits, five guinea-pigs, and four fowls, with negative results.

It is of further interest to note that pathogenic bacteria were not discovered in the flora of the upper air passages of rabbits, guinea-pigs, and fowls, although it frequently happens that these animals perish from sporadic diseases, the lesions of which resemble those produced by the inoculation of the swine-plague germ, and that bacteria which belong to the swine-plague group can be isolated from the affected organs. On the other hand it should be mentioned that cats are immune to large doses of the virluent swine-plague germ.

From the results recorded in the preceding pages it is found that 48 per cent of pigs,† 80 per cent of cattle, 50 per cent of sheep, 16 per cent of horses, 90 per cent of cats, and nearly 30 per cent of dogs contain among the flora of their upper air passages bacteria which possess more or less pathogenic or septic properties. Of these the greater number belong to the swine-plague group, and can be designated as pathogenic bacteria, while the other forms which possess only temporarily destructive power appear to more properly belong to the toxicogenic bacteria. The extremely attenuated forms from the sheep and the great variation found in the pathogenic properties of those from

*Centralblatt f. Bakteriologie u. Parasitenkunde, Bd. XI, 1892, p. 406.

†This is based upon both my own results as recorded in the table and those heretofore published by Dr. Smith.

the cats are interesting in showing the graduation of the swine-plague group of bacteria from the very virulent to the extremely attenuated forms.

The close resemblance of the pathogenic bacteria to the swine-plague germs has been determined by a large number of inoculations and parallel cultures, and by a careful study of their morphology in both fresh and stained preparations. Their feeble power to resist drying and their thermal death point are identical with those of the virulent swine-plague bacteria.

Bacillus No. 1 from cow No. 9 is interesting, from the fact that while it resembles in its general morphological characters the swine-plague germ, it possessed the property of fermenting sugar with the formation of gas. These variations are important in the classification of disease-producing bacteria. The streptococcus isolated from the nares of a healthy horse differed very slightly in its more hardy biological characters, as well as in its effect upon certain animals from the description of the streptococcus which has been described as the supposed cause of strangles.

The very general distribution, in the secretions covering the mucosa of the upper air passages of domesticated animals, of bacteria which possess such marked pathogenic and toxicogenic properties, is of much importance, as it explains the cause of sporadic cases of swine-plague,* and, to some extent at least, the possible source and significance of various toxicogenic bacteria which are frequently isolated from variously diseased animal tissues.

* For a discussion of the relation of these bacteria to isolated cases of pneumonia in swine, see Dr. Smith's Report on Swine Plague, *l. c.*, pp. 109–117.

AN OUTBREAK OF ABORTION IN MARES.

By F. L. KILBORNE.

During the winter of 1890–'91 an outbreak of abortion occurred among the mares of a large stud near Franklin, Pa. On visiting the farm February 5, 1891, I found the stud to comprise about one hundred brood mares and several stallions.

The first mare to abort, due to foal March 21, dropped her foal December 2, or 109 days before full term. Between December 2 and February 5, ten abortions occurred, at periods ranging from 23 days to 195 days, or an average of 100 days, before the completion of the period of gestation. No history of any introduction of the infection from without could be obtained beyond the fact that mares were daily being received at the stock farm from various parts of the State, to be bred to the stallions of the stud. But as far as could be ascertained no mare had been received from any stable in which abortions had previously occurred.

The brood mares were all kept in box stalls, bedded with peat moss, on a dirt floor. The box stalls were arranged in one row around the outside of the barn, and another within. Between the two rows there was a tan-bark track for exercising the animals. The barn was rather damp, but otherwise apparently well suited to the purpose. Each pregnant mare received, on an average, about 6 quarts of oats and a little bran, with a moderate allowance of timothy hay, daily. Mares in poor condition received more, while those in high condition were fed less. The mares were exercised daily, by being ridden on the tan-bark track.

The veterinarian in charge of the stables had taken the usual precaution of removing the aborting mares as soon as discovered, and disinfecting the stall. Such mares were disinfected per vaginum by repeated injections of a diluted solution of corrosive sublimate, 1 part to 10,000 of water, and the external organs, tail and hind limbs sponged over with a stronger solution, 1 part to 1,500 of water. The stable had also been repeatedly fumigated with burning sulphur. Fluid extract of black haw (*Viburnum prunifolium*) in half ounce doses once daily, had been given the pregnant mares during the month preceding February 5.

At the time of the above visit there had been no indications of another abortion during the past week, the last abortion having occur-

red January 29. In order to guard against a continuation of the outbreak, a more thorough system of disinfection of the whole stable was advised and carried out. For this purpose a 2 per cent solution (by volume) of commercial sulphuric acid was used. All stalls were to be disinfected once a week, and any stall occupied by a mare aborting was to be immediately vacated, thoroughly cleaned and disinfected.

The practice of exercising the mares by riding was discontinued, and they were exercised by being led at the halter. On account of the extreme cold weather the drinking water was to be given in quantity not to exceed one-half horse-bucketful at one time, and with the chill taken off. All pregnant mares were to have the tail and external genital organs sponged over daily with a solution of the mercuric chloride 1 part to 1,000 or 1,500 of water. The fluid extract of black haw was continued throughout February, but apparently without result. The mares aborting February 15 to March 4, were among the number receiving the extract.

From January 29 until February 15, no abortions occurred; but from this date until March 4 six mares aborted. A second visit was made to the farm at this time. The additional precautions taken during the past month having failed to check the outbreak, it was deemed advisable to remove all the pregnant mares from the large barn to other stables. Accordingly eighteen of the remaining mares were removed to a stable, formerly used only for colts, with high plank floor and roomy box stalls. The other mares were to be removed as soon as accommodations could be provided for them. Owing to some delay of the carpenters in completing another stable, these mares were not removed from the large barn as intended.

In the barn to which the eighteen mares were removed, all stalls were to be entirely emptied of bedding, and swept and sprinkled with 2 per cent solution of sulphuric acid, twice a week. The disinfection of the large barn was continued as before. All pregnant mares continued to have the root of tail and vulva washed, once daily, with the mercuric chloride solution. In washing, the lips of the vulva were pressed apart with the sponge in order that the solution might be worked in as far as possible. In attempting to inject a diluted solution, even a short distance into the vagina, it was found to produce more or less straining, and was therefore discontinued.

Beginning with March 4, one-half of the mares were to receive the fluid extract of black haw, in doses of one-half ounce once daily; the remaining half of the mares to receive one heaping tablespoonful of the following powder in the feed twice daily, omitting the powders for four or five days after each two weeks: Chlorate of potash, phosphate of soda, of each 1 pound; sulphate of quinine 3 ounces, mix. This treatment was continued to the end of the season. No further abortions occurred.

From the last case to abort, in the evening of March 3, cultures were made on agar-agar early the following morning. The cultures were made by separating the lips of the vulva as far as possible, and lightly

scraping the mucosa of the vulva with a flamed platinum loop and rubbing this upon the inclined agar surface. In these tubes a bacillus appeared, which is described in an article by Dr. Smith, following this account.

Table showing date of abortion and stage of gestation of each mare aborting.

Number of mare.	Due to foal.*	Aborted.	Number of days before close of normal period.	Horse by which mares were in foal.
1	Mar. 21, 1891	Dec. 2, 1890	109	No. 1.
2	Apr. 14, 1891	Jan. 5, 1891	99	No. 1
3	Feb. 13, 1891	Jan. 21, 1891	23	No. 1.
4	Apr. 5, 1891	Jan. 23, 1891	72	No. 2.
5	May 6, 1891	Jan. 23, 1891	103	No. 3.
6	May 1, 1891	Jan. 26, 1891	95	No. 1.
7	Apr. 26, 1891	Jan. 27, 1891	89	No. 1.
8	June 12, 1891	Jan. 27, 1891	136	No. 4.
9	Aug. 11, 1891	Jan. 28, 1891	195	No. 2.
10	Apr. 24, 1891	Jan. 29, 1891	85	No. 1.
11	Apr. 24, 1891	Feb. 15, 1891	90	No. 1.
12	June 2, 1891	Feb. 17, 1891	105	No. 1.
13	May 14, 1891	Feb. 20, 1891	83	No. 5.
14	Apr. 15, 1891	Feb. 24, 1891	50	No. 6.
15	Aug. 25, 1891	Feb. 28, 1891	178	No. 1.
16	July 4, 1891	Mar. 3, 1891	123	No. 2.

*Eleven months allowed as period of gestation.

Of the sixteen mares nine were in foal by stallion No. 1; three by stallion No. 2, and one each by four other stallions.

Mare No. 5 died after the abortion. The others all made a good recovery. No. 7, however, was a chronic aborter, having lost two or three foals previously.

There still remained, March 4, thirty-six pregnant mares, due to foal March 19 to September 14. Two mares due to foal February 18 and 26, making bag. Live foals at term were dropped February 2 and 11, and March 4. One dead foal dropped at term February 19.

In the fall of 1891, when the mares were first brought in from the pasture, three abortions occurred in a small barn where several mares were kept. The aborting mares were at once isolated and the stalls disinfected with the solution of sulphuric acid. The pregnant mares were again given the fluid extract of the black haw in half-ounce doses, once daily, and the vulva and root of tail washed with mercuric chloride solution, as before. No further abortions occurred during the season of 1891–'92.

In order to test the pathogenic properties of the bacillus obtained from mare No. 16, and described by Dr. Smith, vaginal injections were made with pure cultures on one pregnant mare and two pregnant cows. Inoculations were also made on two pigs.

Vaginal injection of pregnant mares.—On April 22 a large bay mare, age 7 years, supposed to be in the ninth month of gestation, received by injection into the vagina 10 cc. of a peptone bouillon culture, two days old, of the bacillus. Twenty-four hours after the injection an abundant, purulent discharge of a grayish-white color was

coming from the vagina. This discharge continued for two days, after which it ceased. The mare developed well-marked symptoms of influenza on the day following the injection, and from April 23 to 30 suffered a severe attack, with a temperature varying from 102.4° F. to 106.2° F. There were several cases of influenza in the stable from which the mare came, so that it is evident the mare contracted the disease before coming to the station. On May 2 the mare dropped a live foal, probably owing to the attack of influenza. The birth was evidently premature, as shown by the lack of development and weakness of the colt, which lived but two days.

Vaginal injection of pregnant cows.—On April 11 a two-year-old heifer, No. 160, and a cow, No. 152, five years old, were chosen for the injection. No. 160 was supposed to be in the seventh or eighth month of pregnancy, while No. 152 was in the fifth month, having taken the bull November 29, 1890. Each cow received by injection into the vagina 10 cc. of a peptone bouillon culture of the bacillus twenty-four hours old. Twenty-four hours after the injection there was quite an abundant, purulent discharge of a dirty grayish-white color from the vagina of both cases. After two days this discharge gradually diminished and had entirely ceased April 15. On April 22 both cows were given a second vaginal injection of a similar culture of two days' growth. No. 152 received 10 cc. as before, and No. 160 received 25 cc. Twenty-four hours after the injection No. 152 showed a slight vaginal discharge, and No. 160 a much more abundant discharge of the same character as before. The discharge had entirely ceased on the third day.

Below is given the temperature of the two cows taken morning and evening during the period of injection:

Date.	Cow No. 160.*		Cow No. 152.	
	9 a. m.	5 p. m.	9 a. m.	5 p. m.
	°F.	°F.	°F.	°F.
April 12	102.8	.....	101.2	.....
April 13	101.2	104.4	101.2	101.6
April 14	104	104.2	101	101.4
April 15	103	102.8	101	102
April 16	103.2	103	101.4	102
April 17	103	103.7	101.4	101.6
April 20	102	103.5	101.4	100.8
April 22	102.2	104	100.8	102.2
April 23	102.6	103.8	101.6	102
April 24	102.7	103	101.6	100.8
April 25	102.5	103.5	101.4	102

*The continued elevated temperature of No. 160 is due to the advanced stage of pregnancy, perhaps. No. 160 drops a healthy calf May 9. No. 152 calves September 5.

Intravenous inoculation of pigs.—April 11, two pigs, five to six months old, numbers 472 and 473, received each by inoculation into the vein on the inside of the thigh, 5 cc. of a peptone bouillon culture twenty-four hours old. On April 12 and 13 both pigs were quite dull and eating but very little. On April 14 to 16 the pigs were active and appetite returning. On April 17 they had apparently entirely recovered.

ON A PATHOGENIC BACILLUS FROM THE VAGINA OF A MARE AFTER ABORTION.

By THEOBALD SMITH.

The two tubes of inclined agar inoculated by Dr. F. L. Kilborne, as described in his report, were handed to me the day following the inoculation. I may repeat here that these tubes were inoculated directly with a platinum loop rubbed over the mucosa of the vulva. They were inoculated in the morning, the abortion having occurred the evening before. In each tube there had appeared, twenty-four hours after inoculation, about a dozen roundish, grayish-white colonies several millimeters in diameter. Some of the growth from each tube seems to have run down the inclined surface of the agar as if semi-liquid. Microscopic examination revealed small rods which did not show any motion when stirred up in sterile water and examined in a hanging drop. Both tubes evidently contained but this one species. From a colony in each peptone bouillon, tubes were inoculated and placed in the thermostat. Both were clouded on the following day and contained short motile rods closely resembling in size and motility hog-cholera bacilli. From both cultures gelatine plates were prepared. On the third day colonies appeared on both sets of plates which were all alike. The bacteria composing them were motile and identical with those in the original tubes. From a colony on a gelatine plate a peptone bouillon culture was prepared, and from this two rabbits were inoculated. The source of the culture may be tabulated thus:

March 5, 1891, agar inoculated from vagina of mare.

|

March 6, peptone bouillon.

|

March 9, gelatine plates.

|

March 12, peptone bouillon (from colony).

|

March 14, two rabbits inoculated.

Rabbit No. 1 received into an ear vein 0.3 cc. of the culture liquid.

Rabbit No. 2 received under the skin of the abdomen the same quantity.

The first rabbit appeared ill on the second day; the inoculated ear slightly swollen and turned back on the head. The same condition prevailed on the third day. Seven days after inoculation it was found dead. As the writer was confined to his bed at this time the animal was examined and the cultures prepared by Dr. V. A. Moore. The spleen very much enlarged, dark, friable. The liver sprinkled with minute areas of necrosis as in hog cholera. In pericardial sac blood-stained serum. The left lung œdematous and some serum in left pleural sac. In cover-glass preparations of both spleen and liver stained with alkaline methylene blue are observed groups of short bacilli. In the blood they are not detected. An inclined agar tube inoculated with a loop of blood showed on the following day about ten nearly round, isolated colonies. They are nearly flat, slightly raised disks from 1½ to 2 millimeters in diameter, of a grayish-white color. An agar tube inoculated with a trace of spleen pulp showed on the following day a large number of isolated and confluent, quite small colonies. The condensation water was covered with a very thin, papery membrane. A peptone bouillon tube inoculated from the spleen at the same time shows, as did the examination of the colonies from the agar tubes, the same motile bacillus which had been injected into the rabbit. The second rabbit remained well until the sixteenth day, when it was unable to move its hind limbs. It was chloroformed at once. No internal lesions were detected. At the place of inoculation slight purulent infiltration of the skin, with enlargement of the neighboring kneefold glands. The spinal cord was not examined.

A second pair of rabbits were subsequently inoculated, the source of the culture used being as follows:

Blood of inoculated rabbit.
|
March 21, agar tube.
|
April 1, peptone bouillon (from colony).
|
April 2, two rabbits inoculated.

Rabbit No. 3 received 0.3 cc. into abdominal cavity.

Rabbit No. 4 received 0.3 cc into an ear vein.

No. 3 did not become ill. Killed on the twelfth day. It showed no trace of local or general infection.

No. 4 died suddenly March 6, four days after inoculation.

The spleen very large, dark and quite firm. A portion of dorsal surface converted into a yellowish necrotic layer. Liver pale. Besides the general parenchymatous changes the tissue was permeated with a network of yellowish necrotic lines. Parenchymatous degeneration of kidneys. Urine turbid; contains very short fragments of granular casts.

Cloudy swelling of myocardium associated with fatty degeneration. Scattered

over ventricular surfaces are yellowish lines parallel to the course of the fibers and about 1 or 2 mm. long. These are made up of necrosed fibers.

Lungs congested and œdematous. Scattered petechiæ over surfaces. One-half of the two small right (cephalic and ventral) lobes hepatized. Hepatized foci in left cephalic lobe.

Blackish, hemorrhagic condition of edge of pyloric valve in duodenum. For several inches below this petechiæ in mucosa. Very many bacteria in preparations from spleen, kidneys, and liver. Few in blood.

From the spleen two gelatine roll cultures prepared. From the blood (right ventricle) agar plates. In all these cultures colonies appeared, to be described more fully below, which were made up of the injected bacteria and these only. At the same time a tube of inclined agar was inoculated with a trace of spleen pulp. On the following day about fifteen colonies of the same bacteria appeared. These inoculations proved the pathogenic character of these bacilli and made it desirable to examine them more carefully.

On gelatine plates or in rolls colonies of these bacilli become distinctly visible to the naked eye about thirty-six to forty-eight hours after sowing. When magnified the deep colonies appear as round, finely granular, brownish disks, which may show later on a more or less distinct peripheral, paler zone. Their size depends on the number of colonies. When about 1 cm. apart they reach a diameter of 0.5 mm. The surface colonies are roundish, slightly convex, whitish, glistening disks with margin slightly wavy or delicately notched. When magnified they show a faintly stippled surface. They become not more than 1 to 1.5 mm. in diameter.

On agar plates the colonies are quite large twenty-four hours after sowing when kept in the thermostat. The deep ones are round or lenticular, opaque with finely knobbed periphery, and 1 mm. in diameter. The surface colonies are flattish, barely translucent, grayish-white disks. When magnified, finely granular in appearance. They may attain a diameter of 4 to 8 mm. when one to two centimeters apart.

Occasionally the deep colonies may become nearly 2 mm. in diameter and the surface colonies now and then spread out so as to attain a diameter of 1 to 2 cm. They then have a smooth, shining, mucus-like appearance, as if the growth had flowed over the surface. In some gas bubbles appear, and occasionally some spread out between the agar layer and the glass beneath.

In tubes of inclined agar, the growth of the isolated colonies is the same as that described for the plates. When confluent a grayish smooth growth is the result. The condensation water becomes turbid.

In tubes of gelatine, the track of the wire appears after two or three days as a thin yellowish-white line, which increases but little in thickness with time. On the surface there is very little growth beyond the point of inoculation.

On the cut surface of boiled potatoes in plugged tubes well supplied with water in the bottom, growth takes place. The potato becomes

covered with a straw-colored growth, the abundance of which depends upon the state of the surface as regards moisture or dryness as well as on the nature of the potato.

In peptone boullion kept in the thermostat there is at first simple cloudiness withir twenty-four hours of inoculation. After some days the liquid becomes more turbid, and a thin cohesive pellicle forms on the surface when the culture tube remains undisturbed.

In milk at 37° C., no change takes place which is visible to the naked eye, even after several weeks' sojourn in the thermostat. Subsequent boiling gives a slightly brownish, more translucent tint to the milk.

The bacilli have the same power as the hog-cholera bacilli of causing fermentation in peptone bouillon to which some glucose has been added. Thus, in bouillon containing 2 per cent glucose, 48 per cent of the volume of the closed branch of the fermentation tube had been occupied by gas in three days. Of this 34 per cent was absorbed by potassium hydrate as CO_2; the rest exploded with air (probably hydrogen). In a recent test with somewhat more alkaline glucose bouillon I obtained the following result:

Fermentation tube inoculated December 30, 1892.

December 31, 10 per cent of closed branch occupied by gas.

January 2, 58 per cent of closed branch occupied by gas.

January 4, 58 per cent of closed branch occupied by gas.

In the temperature of the room the gas occupied only 56 per cent of the closed branch. Of this 37 per cent was absorbed by caustic potash; the rest exploded with air. The culture liquid was markedly acid and free from odor. Gas is not set free in saccharose and lactose bouillon.

In reinoculating this bacillus from time to time into fresh tubes of agar the surface growth assumed a more and more pronounced membranous appearance. The agar was covered with a thin, brittle, somewhat wrinkled layer, which extended down over the condensation water. The same change was observed in bouillon in which a thin papery membrane became a prominent feature of later cultures. This may be replaced by other membranes when shaken to the bottom.

The bacillus under consideration is a short bacillus with rounded ends, which stains well in alkaline methylene blue. It is actively motile in bouillon cultures. It moves to and fro in a straight or curved line, frequently twirling about its own middle point as an axis at the same time. When traces of growth from colonies on agar or gelatine are suspended in water or bouillon, this property of motility may escape attention, since only a few move under these conditions, and in some preparations no motile forms were observed.

The presence of this bacillus exclusively, so far as the agar cultures indicated, in the vagina of a mare which had just aborted, was of considerable moment, and it became necessary to determine—

(1) May injections of cultures of this bacillus produce abortion in healthy pregnant mares?

(2) Is this bacillus present in the genital passages of healthy pregnant and non-pregnant mares?

The first problem was undertaken by Dr. Kilborne, and his notes will be found in the preceding article. They show that an injection of culture fluid into the vagina of one mare and two cows in a state of pregnancy was followed by a discharge, but abortion did not take place.

The second problem was taken up by Dr. V. A. Moore. He failed to find in cultures from one pregnant and four not pregnant mares the pathogenic bacillus just described, as the following brief summary of his observations demonstrates:

"A bacteriological examination was made of the vaginal secretions from five healthy mares. Some of them were on a farm adjacent to the experiment station and some from the stables of a horse car company. One of the mares was pregnant. The method employed was the inoculation of agar tubes with mucus taken directly from the walls of the vagina by means of a sterilized platinum loop. From the colonies of bacteria which developed in these tubes subcultures were made, and from all impure original cultures agar or gelatine plates were made. From mares Nos. 4 and 5 the examination was repeated. The inoculations upon agar directly from the animals were made by Dr. F. L. Kilborne.

The number of cultures that were made and the bacteria that were obtained from the different animals are given in the subjoined table:

Mare No.	Number of cultures from vaginal secretions.	Date of cultures.	Number of tubes that remained clear.	Number of species.	Remarks.
	Agar.	1891.			
1	2	April 7	2		
2	1	...do		5	2 bacilli, 2 micrococci, 1 streptococcus.
3	2	April 26		2	2 streptococci.
4	2	May 5	1	1	1 streptococcus.
	2	June 10	2		
5	2	May 5		2	1 micrococcus, 1 streptococcus.
	2	June 10	1	2	1 micrococcus, 1 streptococcus.

It should be stated that the cultures from mare No. 3, and the first cultures from No. 5, were pure. The streptococcus from the second set of cultures from No. 5 was not distinguishable from one of the streptococci from mare No. 3. The large number of bacteria from No. 2 and the comparatively small number obtained from the other animals is worthy of note. It will also be observed that streptococci were universally present in the cultures. In all, eleven apparently different species of bacteria were isolated. They were classified as follows: Two non-motile bacilli, four micrococci, and five streptococci. The streptococci were very delicate, and the differences that were found to exist between them were very slight. The micrococci were more saprophytic in their biological characters, and were readily differentiated. One of bacilli the was club-shaped, and resembled morphologically, and in its

cultural characters, the pseudo-diphtheria bacillus. The other bacillus was slender and usually united in chains. It was aërobic and did not ferment glucose."

The results thus far obtained, while they do not demonstrate the causal relation between this pathogenic bacillus and the abortion, nevertheless do not disprove such relation. The negative outcome of the injection experiments should not be rated too high in this connection, for there may be other as yet unknown factors necessary in order that the infectious agent may bring about abortion. It is true that we know nothing as yet concerning the pathological processes involved in epizoötic abortion, and it would be premature to claim that some living organism like a specific bacterium is responsible for it. There may be other causes at work of which we have no information. Yet, from the standpoint of to-day, the assumption of living organisms, such as bacteria, explains best the occurrence as related above, and the actual presence of a pathogenic bacillus in the vagina of a mare after abortion is a fact worthy of more attention than we have been able to give it thus far.

Another fact of interest in connection with this pathogenic bacillus is its close resemblance to the hog-cholera bacillus. As I shall show by illustrations in a future article, the specific bacillus obtained directly from outbreaks of hog cholera varies slightly in its biological and morphological characters, and if the bacillus in question had been sent to me as having come from swine I should not have hesitated to regard it as a hog-cholera bacillus of rather feeble virulence, and possessing some slight differential characters from the virulent form. In other words, the differences between this form and the virulent hog-cholera bacillus are no greater than between the hog-cholera bacilli of widely varying pathogenic power which I have thus far investigated. This fact does not of course prove it to be identical with *Bacillus cholerœ suis*, but the presumption is strongly in favor of a close relationship. The points of resemblance include form, motility, growth on the various culture media, including the fermentation tube, staining reactions, and pathogenic action. The only points of difference are feebler pathogenic action and a tendency to a membranous growth on agar, which tendency, however, is not pronounced.

Granted the close relationship of this bacillus with the bacillus of hog cholera, it is not safe to infer that abortion in mares stands in a causal relation to hog cholera or the reverse, and yet such relation is by no means disproved by the foregoing investigations. All that we may safely affirm is that there is evidently a wide distribution of bacteria closely related to the hog-cholera bacillus, including such bacteria as *B. enteriditis* Gärtner, *B. typhi murium* Löffler, and the bacillus under discussion. This possible relationship brings up the important question whether certain specific infectious diseases of any given species of domesticated animals may appear also in other species, but un-

der a different form, and therefore only identifiable by a study of the bacteria or other microörganisms which produce them. May we, in other words, have diseases among domesticated animals known under different names and yet due to the same cause? Pending the solution of such problems by a thorough investigation of all communicable diseases among domesticated species and species of wild animals coming in contact with them, we would urge owners and breeders of animals not to take it for granted that diseases, even among different species of animals, are necessarily different until such has been demonstrated to be the case by investigation. In other words, the indiscriminate mingling of sick and healthy animals may be really more dangerous than we are in the habit of regarding it.

The results of this investigation may be briefly summarized as follows:

1. The discovery of a pathogenic bacillus in the vagina of a mare soon after abortion.

2. The very close relationship between this bacillus and the hog-cholera bacillus.

3. The production of a slight discharge by the injection of cultures into the vagina of a pregnant mare and two pregnant cows.

4. The absence of this bacillus from the genital passages of one pregnant and four non-pregnant mares.

SOME EXPERIMENTAL OBSERVATIONS ON THE PRESENCE OF TUBERCLE BACILLI IN THE MILK OF TUBERCULOUS COWS WHEN THE UDDER IS NOT VISIBLY DISEASED.

By THEOBALD SMITH and E. C. SCHROEDER.

The possible dangers to health arising from the consumption of meat and milk from tuberculous cattle were discussed by one of us in a former report,* and the state of the subject summarized from the results of experiments made by various observers up to that time. Since then a small number of milch cows which were affected with this disease have come under observation in the District of Columbia. It was deemed advisable to take up this subject and determine if possible the extent of milk infection and its relation to the general condition of the animal. Only a small number of cases have been experimented on, but the facts are published now, inasmuch as they have accumulated very slowly and are not likely to be collected any more rapidly in the future.

The tuberculous animals were taken to the Veterinary Experiment Station where they were in charge of F. L. Kilborne, B. V. S., to whom we are indebted for some of the clinical notes. The two cases reported first have been already briefly referred to in a former report.† To determine the presence or absence of tubercle bacilli a certain quantity of milk, in the later cases centrifugalized, was injected directly into the abdomen of guinea-pigs. These were kept under observation a certain length of time before being killed for examination.

No. 155, Jersey cow, seven years old, from a large herd in the District of Columbia. Tuberculosis diagnosed, and the animal transferred to the Experiment Station February 28, 1891.

There was at this time some emaciation, which became more marked in the following months. The animal lived until June 24, on which day she was found lying down in an insensible condition, and was dispatched by a blow on the head. At the autopsy advanced caseous masses were found in both lungs as well as in the bronchial and mediastinal glands, which were enormously enlarged.

* Report of the Secretary of Agriculture for 1889, p. 104.

† Report of the Secretary of Agriculture for 1891, p. 135.

Guinea-pigs were inoculated as follows:

No. of animal.	Weight.	Quantity of milk injected.	Date of injection.	When killed.	Weight when killed.	Remarks.
	Ounces.	*C C.*	1891.	1891.	*Ounces.*	
1.....	14.5	7	Mar. 18	May 12	20	No tuberculosis.
2 (male).....	13	7	...do	July 29	33	Do.
5 (female)...	11	5	Apr. 4	...do	20	No tuberculosis; animal nursing one young.
6 (female)...	12.5	5	...do	...do	25	No tuberculosis.
9 (female)...	.....	7	June 22	Oct. 10	27	Tuberculosis.
10.....	24	7	...do	...do	30	No tuberculosis.

This table shows the absence of tuberculosis in all but one case, which had been inoculated with milk (together with one other) only two days before the cow was in a dying condition, and when the secretion had nearly ceased.

The tuberculous lesions in this guinea-pig are of interest. The anterior mediastinal glands at the root of the thorax were quite large and contained calcareous foci. There were a small number of subpleural tubercles. The spleen, though not much enlarged, contained bunches of tubercles projecting slightly above the surface, and giving the organ a nodular appearance. A few necrotic foci were found in the liver. The guinea-pig was quite large and fat when killed, and no one would have suspected tubercular infection.

These inoculations show that even though the cow was emaciated the milk did not contain tubercle bacilli until shortly before death, and even then only one out of two inoculated guinea-pigs became infected.

No. 156, Jersey cow 8 or 9 years old, from the same herd as No. 155. Tuberculosis diagnosed February 28, 1891, and the cow transferred to the experiment station on this day. Emaciation had already set in and continued, so that at the time of her death, June 5, 1891, she was a mere skeleton.

There were extensive tuberculous growths on the omentum and in the peritoneum covering the paunch, the diaphragm, the abdominal walls, the spleen, kidneys and liver. The mesenteric, portal, and renal glands were transformed more or less into caseous masses. Of the abdominal organs there were tuberculous foci in the liver and the kidneys. Those in the kidneys were few in number and calcified. The Fallopian tubes and the lining membrane of the uterus contained very extensive cheesy deposits. In the thorax, the lungs were very extensively involved in tuberculosis, similarly all the lymph glands and the costal and pulmonary pleura. There were large deposits on the epicardium. The infection of the glands in the muscular tissue was not specially searched for. It should be mentioned, however, that there were no enlarged glands visible in any portion of the body, and that the axillary glands were normal. The udder was free from all changes visible to the naked eye.

Only four guinea-pigs were inoculated with the milk of this cow.

No. of pig.	Weight.	Date of injection.	Quantity injected.	When killed.	Weight when killed.	Remarks.
	Ounces.		*CC.*		*Ounces.*	
3 (female)	12.25	Mar. 18	7	May 12	14	No tuberculosis.
4 (female)	13.25	... do....	7	July 29	*16	Do.
7 (male)	9.50	Apr. 4	5	July 29	23	Do.
8 (male)	12.25	...do	5	... do....	27	Do.

*Nursing three young.

The milk from these cows was used to feed two pigs as long as it lasted. This was done by Dr. Kilborne, who has furnished the following notes concerning the feeding:

Pig No. 489.—Female, 2 months old on April 16, 1891; weighed 14 pounds.

Pig No. 490.—Female, 2 months old on April 16, 1891; weighed 16½ pounds.

On April 16 the feeding was begun. Each pig received daily about 1 gallon of milk in two meals until May, when the quantity began to decrease. On June 5, No. 156 died and No. 155 furnished about 1 quart daily until June 20, when the milk gave out.

Both pigs were killed July 29, 1891. At this time No. 489 weighed 63 pounds, and No. 490 78 pounds. Both were in excellent condition and the organs free from disease.

No. 233, Jersey cow, 7 years old. Tuberculosis diagnosed, and the animal sent to the Experiment Station August 31, 1892. At this time she was quite thin and coughed more or less. Enlargement of subcutaneous lymphatics not discoverable.

Up to October 5 she was milked once a day, and on this day she gave only about a pint. She was failing rapidly, and on October 11 she was found dead.

At the autopsy tuberculous processes were observed in the following organs and localities:

In the cortex of one lymph gland behind udder a few quite small tubercles with yellowish center. The omentum over paunch studded with a large number of confluent tubercles; similarly the abdominal surface of the diaphragm. The capsule of liver and spleen was studded with tubercles, but the parenchyma of both organs was free. The mesenteric and portal glands extensively necrosed. Through the parenchyma of kidneys were disseminated cheesy tubercles from 1 to 2 inches apart, and about one-eighth inch in diameter.

Extensive tuberculous disease of the Fallopian tubes and ovaries, as well as of the mucous membrane of the uterine horns.

In the thorax the anterior and posterior mediastinal glands, as well as the bronchial glands, are greatly enlarged and converted in part into caseous masses. Through the parenchyma of both lungs are disseminated caseous foci of various sizes, while the small lobes of the right lung (cephalic and ventral lobes) are completely transformed into caseous masses. The pleura over these lobes is thickly studded with tubercles.

The milk was tested a short time before the death of the animal, and although the udder contained, so far as the naked eye could tell, no foci of disease, the secretion contained tubercle bacilli, as the following table demonstrates:

No. of guinea-pig.	Weight.	Date of injection.	Quantity of milk.	When killed.	Weight when killed.	Remarks.
	Pounds.	1892.	*CC.*	1892.	*Pounds.*	
302	1.25	Oct. 4.	5	Dec. 24.	1.343	No tuberculosis.
303	1.18	....do	5	....do	1.343	Do.
304	1.18	....do	5	....do	1.25	Tuberculosis.
305	1.5	....do	5	....do	1.314	Do.
306	0.95	....do	5	....do	1.047	Do
309	1.37	Oct. 8.	5	....do	1.25	Do
310	1.5	....do	5	....do	1.156	Do.
311	1.18	....do	5	....do	0.953	Do.
312	1.5	....do	5	....do	1.375	Do.
313	1.43	....do	5	....do	1.265	Do.

NOTE.—Nos. 302 to 306, inclusive, received milk from left forequarter only. Only about 50 cc. could be drawn at the time. Nos. 309 to 313, inclusive, received milk from the other quarters mixed.

Of the first five cases three were found tuberculous. Of the second lot of five all were tuberculous. The first set had been inoculated with milk drawn from the udder seven days before death; the second set with milk drawn only three days before death. There was, therefore, a distinct increase in the number of bacilli in the milk as death approached. That the animal was perhaps going through the earliest stages of an acute generalized miliary tuberculosis affecting the udder as well as the other organs of the body was suggested in the microscopic examination of a fresh section of liver tissue, in which a group of three giant cells came clearly into view. Sections of hardened tissue have not been examined.

As to the character of the tuberculosis affecting the guinea-pigs the following general remarks will suffice:

1. Marked enlargement of the lymph glands of abdomen, especially the one behind (or dorsad of) the stomach, and of those at root of thorax (anterior mediastinal).

2. Puriform, semi-fluid condition of the contents of these glands excepting in one case in which they were still firm.

3. In two cases the subcutaneous glands were affected.

4. Marked nodular condition of the spleen owing to agglomerations of tubercles within its substance.

5. Lines of retraction of the convex surface of the liver with very few distinctly tuberculous lesions.

6. Tubercles of the omentum in several cases.

No. 234, Jersey cow from the same herd as No. 233. Sent to the Experiment Station August 31, 1892, with the latter. Tuberculosis suspected from physical signs. Positive reaction with tuberculin at two different times. She was killed February 6, 1893, in a condition of marked emaciation. Of the autopsy notes, the following may be quoted:

Udder, together with lymph glands, normal.

In the thorax, the caudal half of both principal lobes firm, nodular to the touch.

On section tuberculous foci of various dimensions from miliary tubercles up to caseous foci fully three-fourths of an inch in diameter. The bronchi contain an abundance of muco-pus. Scattered masses of tubercles on the pleura of the diseased areas and on corresponding costal pleura. Bronchial and mediastinal glands very large with caseous contents.

In abdomen the mesenteric glands are very large and transformed into caseous masses which contain calcareous particles. In the capsule of the liver three cheesy nodules as large as peas. Parenchyma of this organ as well as of spleen and kidney free from tuberculosis, although the gland at the hilus of the latter is enlarged and partly caseous.

The milk from this cow was centrifugalized before injection into guinea-pigs. The small Litten hand-centrifugal machine was used for this purpose. According to Scheurlen* bacteria do not behave alike under the influence of this process. Anthrax bacilli and their spores, the bacilli of typhoid fever and Asiatic cholera, gather in the cream, while tubercle bacilli are thrown down. In the same way tubercle bacilli tend to settle when milk is allowed to stand for some time in the refrigerator. Basing our procedure on these determinations we retained the milk in the bottom of the tubes after centrifugalizing and rejected the rest. The glass tubes which were to hold the milk during centrifugalizing were sterilized dry beforehand. The milk drawn as heretofore directly into cotton-plugged, sterilized 6-ounce wide-mouthed bottles, was distributed into the four tubes of the centrifugal machine and revolved for forty-five minutes at a rate of 1200 revolutions per minute. The milk and cream at the surface was then carefully withdrawn with a sterile pipette and about 0.5 cc. in the bottom retained.

The following table gives in brief the experimental inoculations with the milk from No. 234:

No. of guinea-pig.	Weight.	Date of injection.	Quantity injected.	Centrifugalized from.	Guinea-pig killed.	Weight when killed.	Remarks.
	Pounds.		*CC.*	*CC.*	*1893.*	*Pounds.*	
359	.....	Dec. 23, 1892	1	40	Mar. 25	.....	No tuberculosis.
355	1.06	Dec. 24, 1892	6	40	Mar. 10	1.11	Do.
362	1.09	Dec. 28, 1892	4.5		...do	1.06	Do.
360	1	Jan. 10, 1893	5.5	80	April 13	1.5	Do.
361	1.5	....do	6		...do	1.28	Do.
263	1.125	Jan. 11, 1893	5.5	100	...do	1.28	Do.
264	.....	....do	5,5		...do	.....	Found dead of pneumonia 5 days after inoculation.
272	1.125	Jan. 28, 1893	6	60	...do	1.25	No tuberculosis.
373	1	....do	6		...do	0.93	Do.

In almost every case the milk was allowed to stand over night in the ice chest, and only the lower strata centrifugalized. In this way both sedimentation and centrifugalizing came into play. Granted that the latter process acts as determined by Scheurlen, the milk of this cow, even in the far advanced stage of tuberculosis, was free from tubercle

* Ueber die Wirkung des Centrifugirens auf Bakteriensuspensionen besonders auf die Beurtheilung der Bakterien in der Milch. Arbeiten aus dem Kaiserl. Gesundheitsamte, VII, 1891, 269.

bacilli. Cover-glass preparations of the sediment, prepared January 28, were carefully examined for tubercle bacilli, but only a small number of multinuclear leucocytes were detected.

Cow No. 303.—The milk from this animal was tested but once. On December 8, 1892, 60 cc. was centrifugalized for thirty minutes. In a little of the sediment stained, multinuclear leucocytes were detected, but no tubercle bacilli; 6 cc. of the milk in the bottom of the tubes was injected into the abdominal cavity of guinea-pig No. 356. It was killed with chloroform March 25, 1893, and found healthy.

The cow was killed March 18, 1893, a little more than three months after the milk was tested. Tuberculous foci partly caseous, partly calcareous, were found in the lymphatic glands behind the udder; about half a dozen tuberculous foci in the lungs, one as large as a hen's egg. The bronchial, mediastinal, mesenteric and portal lymph glands contained tuberculous masses.

Cow No. 314.—The milk of this animal was tested at the same time. The guinea-pig inoculated with 6 cc. of centrifugalized milk was killed with the preceding, three and one-half months after the inoculation, and a little grayish, pneumonic infiltration of one lung discovered. Tuberculosis not detected.

The cow was killed March 18, 1893. The udder and corresponding lymph glands were free from disease. Tuberculosis of the lungs was present in the form of several foci, one 4 by 2½ inches in size. The bronchial and mediastinal glands were considerably involved; the portal and mesenteric glands only slightly so.

The facts thus far obtained may be most conveniently put into the form of a table in which the period of injection of the milk is outlined by the last negative and first positive results:

No. of cow.	Tubercle bacilli present.	Tubercle bacilli absent.	Condition at autopsy of—		Condition of animal at time of death.
			Udder.	Udder glands.	
155	2 days before death.	2½ months before death.	Normal	(?)	Tuberculosis very extensive.
156	.....	2 months before death.	....do.....	(?)	Do.
233	6 days before death.	.....	....do.....	Slight infection...	Do.
234	.....	9 days before death.	....do.....	Normal	Do.
303	.....	3½ months before killed.	....do.....	Tuberculous.....	Tuberculosis moderate.
314	.....	....do	....do.....	Normal	Do.

These determinations show, as was suggested in the article referred to above, that if a rigid inspection of all dairy herds be enforced so that all animals showing any affection whatever of the udder, or any emaciation, could be at once removed from the herd and the milk condemned,* perhaps most of the infected milk would be thereby excluded. This inspection does not settle the serious question of bovine tuberculosis in

*This should be done even if the reaction with tuberculin proves negative.

its relation to human health. It would, however, be a great stride toward the settlement of this question, pending a more accurate determination of the extent of the disease. The use of tuberculin and the more careful examination of the organs at the abattoirs in Europe is revealing a much higher percentage of tuberculous cattle than had been heretofore even suspected, and we shall soon be prepared to publish additional interesting facts and deductions bearing on this important matter.

ADDITIONAL OBSERVATIONS ON TEXAS CATTLE FEVER.

By THEOBALD SMITH, F. L. KILBORNE, and E. C. SCHROEDER.

ON THE INDEFINITE PERSISTENCE OF THE TEXAS-FEVER PARASITE IN THE BLOOD OF SOUTHERN CATTLE.

Since the preparation of Bulletin No. 1 of the Bureau of Animal Industry on Texas cattle fever, several additional facts have been discovered concerning the peculiar nature of this disease which we deem of sufficient importance to place on record.

In the bulletin referred to (p. 119) it was shown that the blood of Southern (North Carolina) cattle contained the micro-parasite of the disease some time after such cattle had left the permanently infected territory, and after they had been rid of cattle ticks. In the final experiment recorded, the blood of a Southern cow (No. 214) was still capable of producing disease in natives when injected into a jugular vein seventy-four days after the animal had left the permanently infected territory, and about fifty days after the last ticks had been removed.

In continuation of the same investigations another native (No. 211) was inoculated with the blood of the same Southern cow (No. 214) on October 13, 1892, or one hundred and eight days after the latter had left North Carolina pastures. The native was a tuberculous cow in fairly good condition. An enormously enlarged lymphatic gland was situated behind the angle of the lower jaw. The inoculation was made as in previous cases, the blood being drawn from the jugular of No. 214 with a large hypodermic syringe and injected directly into the jugular vein of No. 211. The following table and notes give the subsequent history of this case:

No. 211.

Date.	Number of red corpuscles.	Parasites in red corpuscles.		Remarks.
		In fresh preparations.	Dried and stained.	
Oct. 13, 1892	5,678,500 (white 7,142).	5 per cent bright bodies; size variable; some motile.	None	Jersey heifer, 2 years old, received April 20, 1892, affected with tuberculosis; has a large gland behind lower jaw on left side; expiration and inspiration accompanied by a grunting sound; expiration much prolonged. Receives into left jugular 28 cc. (2 syringefuls) of fresh blood drawn directly from cow No. 214.

No. 211.—Continued.

Dates.	Number of red corpuscles.	Parasites in red corpuscles.		Remarks.
		In fresh preparations.	Dried and stained.	
Oct. 18, 1892	5,250,000 (white 13,750).	15 to 20 per cent bright bodies up to $\frac{1}{2}\mu$ in diameter; 4 or 5 in some corpuscles.	None.	
Oct. 22, 1892	4,304875 (white 4,875).	About 3 per cent large parasites; some change their outline.	Both paired and single parasites.	
Oct. 24, 1892	2,677,000 (white 5,555).	Many bright bodies; 1 to 2 per cent large parasites; many in pairs and pyriform.	Same as in fresh preparations.	Appetite partly lost.
Oct. 25, 1892	.....	5 to 10 per cent bright bodies; large parasites very scarce.	None.	Refuses all food; lies down most of the time; losing flesh rapidly.
Oct. 27, 1892	.....	.....	.....	Found dead this morning; no hæmoglobinuria observed during the fever, although the animal was carefully watched.

The temperature of this animal began to rise October 14, reaching its highest point (105.8) October 18.

AUTOPSY NOTES.

Animal in good condition as regards fat. Weighs about 550 pounds. Hæmorrhagic discoloration of the left superficial jugular vein extending from the place of injection to the root of the neck and toward the head. On the left side, resting against the larynx, a fluctuating gland fully 4 inches in diameter. It is readily dissected out, and when incised the thickened capsule is found to inclose a turbid fluid holding caseous masses in suspension. Near this gland is a smaller one with contents completely caseous.

Heart.—Usual ecchymosis under epicardium and under endocardium of left ventricle. Both ventricles contain dark coagula of moderate size. Parenchymatous changes of heart muscle very slight. Lungs not collapsed. Subpleural emphysema over portions of smaller anterior lobes. Red hepatization of a portion of the right ventral, of a contiguous portion of the right principal, and of the entire azygos lobe. The two first-mentioned lobes are interspersed with small necrotic foci. The whole emits an offensive odor. The portion of diaphragm nearest the hepatized azygos lobe is sprinkled with hemorrhages. The lung contains some tuberculous foci as follows: A soft cheesy mass, 1 inch in diameter, in the left cephalic, a similar one in the right principal lobe, and a smaller one in the hepatized region of the right ventral lobe. Bronchi of hepatized regions intensely dark red; occasionally clumps of yellowish masses encountered. The pneumonia is probably due to a recent discharge of a caseous mass into a bronchus, resulting in aspiration pneumonia of a septic character.

Spleen.—Weighs 5¼ pounds, 25 inches long, 8 wide, and 1¼ thick. More or less extravasation of blood from vessels of capsule. Usual markings of pulp effaced by the great engorgement with blood corpuscles.

Liver.—Weighs 14½ pounds without gall bladder. Color paler than normal. The surface sprinkled with closely set irregular grayish spots about ½ mm. in diameter. On section the liver presents a decidedly yellowish appearance. Much thick blood flows from hepatic vessels. The grayish points not visible on the cut surface. In fresh sections occasional areas of bile stasis noticed and much fatty degeneration of the parenchyma. Intracellular pigment abundant.

Bile.—(8 ounces) is very highly charged with flakes, so that it scarcely flows.

Kidneys.—Much paler than normal. On the surface asm all number of grayish spots corresponding on section to grayish cylindrical lines passing through the cortex and indicative of fatty changes. Similar radial lines on pyramidal portion corresponding to tubules filled with fat globules. Much pigment in the form of granules up to 0.5μ in diameter in the epithelium of cortex and medulla. Papillæ more or less reddened and the inclosing calices are ecchymosed.

Urine.—Pale yellow; contains a trace of albumen.

Digestive tract.—Slight bluish-pink discoloration of mucosa of fourth stomach. Mucosa of small intestines pale and covered with a pasty, bile-stained layer of desquamated epithelium.

Uterus.—Contains a fœtus 10 inches long.

The number of infected corpuscles was quite small at this stage of the disease. In the capillary blood from the heart none were seen. In the liver and spleen only a few were detected. In kidney preparations from 1 to 2 per cent were present. They are large, roundish, mainly single. A small number of tinted macrocytes are present in the various organs.

The following case is of unusual interest, for it demonstrates that the Texas fever parasite may remain an indefinite length of time in the blood of insusceptible animals.

No. 113, a cow brought from North Carolina in September of 1889, and at that time 3½ years old, has been kept on the Station since that time. She has not been exposed in fields infected with ticks or Southern cattle or sick native cattle. She has, in other words, not come in contact with the Texas fever infection in any way.

On October 29, 1892, a native cow, No. 235, which had been on the Station during the past two years, was inoculated with the blood of No. 113 in the way indicated in the preceding case. A syringeful of blood (14 cc.) was withdrawn from a jugular vein of No. 113 and injected at once into a jugular of No. 235. This was repeated, hence the latter animal received 28 cc. in order that the experiment might be a precise parallel to similar ones made earlier in the season.* The following tabulated record demonstrates the presence of Texas fever:

No. 235.

Date.	Number of red corpuscles.	Parasites in red corpuscles.		Condition of red corpuscles.	
		In fresh preparations.	Dried and stained.	In fresh preparations.	Dried and stained.
Oct. 29, 1892	6,375,000 (white 10,000).	Some bright bodies.	None.....	Normal.....	Slight variation in size.
Nov. 9, 1892	5,050,000 (white 6,250).	Negative	Several irregular and one pair pyriform parasites.	....do	Do.
Nov 12, 1892	3,815,555 (white 7,700).	A few bright bodies.	Several parasites of irregular outline.	....do	Normal.
Nov. 14, 1892	4,287,500 (white 7,500).	....do	None.....	....do	Do.
Nov. 19, 1892	5,575,000 (white 10,000).	....do	....do.....	5 to 10 per cent macrocytes up to 8μ in diameter.	Same as in fresh preparations.
Nov. 30, 1892	4,700,000 (white 18,750).	(Preparation poor.)	Negative.....	A few macrocytes.	A few macrocytes.

NOTE.—The temperature in this case was high from November 6 to November 13.

*Bulletin No. 1, p. 119.

As a parallel to the preceding inoculation the following may be cited here, although it does not bear directly on the subject under discussion:

On August 27, 1892, defibrinated blood from a case of Texas fever* collected with all precautions necessary in bacteriological work, was drawn up into sterile pipettes and some placed at 36° C., others in a dark receptacle at the ordinary temperature. These pipettes remained undisturbed until November 3, when the blood was withdrawn from all and mixed together, making about 7 cc. It was free from bacteria. This blood was injected into a jugular vein of No. 236. The record below shows that no disease was produced. The blood at the time it was drawn and after a few days' standing in a cool place produced Texas fever in four cases inoculated therewith.†

No. 236.—Cow, 5 years old, received October 29, 1892, from Maryland.

Dates.	Number of red corpuscles.	Parasites in red corpuscles.	
		In fresh preparations.	Dried and stained.
Oct. 29, 1892	6,609,370 (white 10,937)	Some bright bodies	None.
Nov. 9, 1892	6,737,500 (white 13,750)	do	Do.
Nov. 14, 1892	6,093,000 (white 6,666)	do	Do.
Nov. 19, 1892	6,475,000 (white 7,500)	do	Do.

NOTE.—The temperature of this case at no time rose above 102.6° F.

The following severe case of Texas fever, followed by relapse, was produced in midwinter by inoculation with blood from a North Carolina cow:

No. 237, a native cow, 5 years old, from Prince George County, Md., was inoculated January 12, 1893. Twenty-eight cc. of blood, drawn with a hypodermic syringe from a jugular vein of North Carolina cow No. 214, was injected directly into a jugular vein of this cow. The fever appeared in five days, and the destruction of the red blood corpuscles was well under way on the seventh day after inoculation, as the table below shows:

No. 237.

Dates.	Number of red corpuscles.	Parasites in red corpuscles.		Remarks.
		In fresh preparations.	Dried and stained.	
Jan. 10, 1893	5,962,500 (white 10,000).	2 to 3 per cent bright bodies.	None	
Jan. 12, 1893				Injection made to-day.
Jan. 19, 1893	4,287,500 (white 10,000).	A few bright bodies; 1 per cent amœboid and paired parasites.	One-half per cent large, single and paired parasites.	
Jan. 21, 1893	3,561,600 (white 42,465).	Parasites very scarce.	Several large parasites in each preparation.	Appetite failing.
Jan. 25, 1893	2,314,000 (white 4,271).	do	Very few parasites	January 26. Gave birth to a calf last night; has lost considerable flesh and is very weak.

* Bulletin No. 1, p. 81. † Loc. cit.

No. 237.—Continued.

Dates.	Number of red corpuscles.	Parasites in red corpuscles.		Remarks.
		In fresh preparations.	Dried and stained.	
Feb. 17, 1893	1,851,300 (white 4,054)	None	Some peripheral cocci.	Relapse beginning.
Mar. 1, 1893	1,033,000 (white 1,515).	Some pale peripheral bodies.	5 per cent peripheral cocci.	
Mar. 20, 1893	1,246,000 (white 6,350).	Some bright bodies .. Some pale peripheral bodies.	Some peripheral cocci.	
Mar. 31, 1893	2,170,000 (white 6,154).	A few bright bodies .	Very few peripheral cocci.	

NOTE—The temperature in this case remained high (103.5–105.5) almost continuously from January 17 to January 25.

ON THE PERSISTENCE OF THE TEXAS-FEVER PARASITE IN THE BLOOD OF INOCULATED NATIVE CATTLE.

That the Texas-fever parasite might persist in the blood of susceptible cattle long after recovery from the disease did not suggest itself until this interesting fact was accidentally discovered.

In 1891 several animals which had been inoculated with blood from a case of Texas fever passed through a severe attack of this disease.* Two of these were reëxposed the following summer.

Just before the exposure on the infected pasture the customary blood examination was made, and it was found that in one of these animals the Texas-fever micro-parasite was present, though in very small numbers. The peculiar pyriform bodies in pairs within the red corpuscles left no doubt as to their nature. Inasmuch as this animal had not been exposed to the infection since the inoculation, the only conclusion which could be reached was that the micro-parasite had persisted in the blood since last fall. This persistence, astonishing in itself at the time, is now quite in harmony with the long life of the species in the blood of Southern cattle.

Another fact of interest is the insusceptibility on the second exposure of the animal in which the micro-parasite was found. The other animal, whose blood on microscopic examination no longer revealed the micro-parasite, was not insusceptible, for it passed through a well-marked but mild attack.

ON PREVENTIVE INOCULATION.

The foregoing observations furnish an important basis for a rational method of protective inoculation with reference to Northern cattle about to enter the Southern permanently infected territory. We have shown in the foregoing pages and in the bulletin on Texas fever that the blood of Southern cattle contains the micro-parasite of Texas fever an indefinite length of time, and that every intravenous inoculation with such blood has produced the disease in natives. Moreover, the disease induced by a single inoculation of such blood is by no means so

*Bulletin No. 1, pp. 80 and 134; also Nos. 182 and 185 in the appendix.

fatal as the natural disease. In fact, we have recorded but one death in midsummer due to inoculation. A third important consideration is the production of the inoculation disease at all seasons, even in midwinter.

Putting these facts together we are led to the inference that, inasmuch as a well-marked attack of Texas fever will probably protect from a future fatal attack, the first attack might be induced artificially in fall or winter by direct inoculation. In other words, the protective inoculation would be a real attack of the disease. It is not likely that, even in older cattle, such inoculation would result fatally in late fall or winter. Of inoculation with the blood of sick natives we can not make such positive statements, since we have had a considerable percentage of deaths in September from such inoculations.

In the bulletin on Texas fever it was suggested that native cattle might be exposed to a mild form of the disease by scattering ripe, egg-laying ticks on an inclosed pasture upon which the cattle to be exposed are confined. While we have found nothing thus far to invalidate this method, we nevertheless feel that the method of intravenous inoculation can be made more certain by some additional experimenting. It is desirable to determine, also, whether the simple subcutaneous injection of blood from Southern cattle will suffice to induce Texas fever. The subcutaneous injection of defibrinated blood taken from natives during the fever was followed by positive results (in one old cow, by death). The much larger number of micro-parasites in such blood will perhaps account for the very positive results obtained.*

It should be stated that preventive inoculation is of no avail during the prevalence of the disease, owing to the long duration of the disease and the consequent slow acquisition of immunity.

In conclusion, the important facts in this article may be very briefly summarized as follows:

1. The indefinite persistence of the micro-parasite of Texas fever in the blood of Southern cattle after removal from the enzoötic territory.
2. The persistence (for nearly a year) of this micro-parasite in the blood of an inoculated native.
3. The production of a severe case of this disease in midwinter by inoculation.
4. The destruction of the micro-parasite in a quantity of defibrinated blood kept sixty-eight days before it was tested by inoculation.
5. The probable development of a simple method of preventive inoculation by the use of blood from Southern cattle.

* Since this was written, subcutaneous inoculation with blood from Southern cattle has been found equally efficacious. The intravenous injection thus becomes unnecessary.

PRELIMINARY NOTES ON A SPOROZOÖN IN THE INTESTINAL VILLI OF CATTLE.

By THEOBALD SMITH.

[Plate I.]

In the summer of 1889, during the examination of cattle which had succumbed to Texas fever, I observed on the mucous membrane of the small intestine in its lower portion minute white bodies of a spherical outline which were just visible to the naked eye. Several observers, even when informed of their presence in a given portion of the mucous membrane, passed them by at first until they were directly pointed out. They may, therefore, pass unnoticed, unless the scrutiny of the mucosa is sharp. When the membrane is stroked with the back of the scalpel it will be noticed that these bodies change their place slightly without becoming detached, indicating that they are located within the villi near their free end. This supposition is confirmed by carefully removing a number of villi, where such a body is found, with small forceps, and examining them under a low power. It will then be seen that an opaque round or slightly oval body is situated within one of them directly under the epithelial layer. The removal is accomplished best by clasping at their roots the villi in the immediate vicinity of such a body with delicate forceps and gently pulling upward. The parasite comes away intact, and is easily teased out of the adherent cell mass without rupture of the cyst. These bodies vary considerably in size, and are either spherical or ovoid. Of a number of measurements of bodies of the larger varieties the following may be quoted:

(1) 0.416^{mm} long, 0.364^{mm} broad.
(2) 0.312^{mm} long, 0.208^{mm} broad.
(3) 0.312^{mm} long, 0.312^{mm} broad.
(4) 0.364^{mm} long, 0.312^{mm} broad.

It is probable that the weight of the cover glass may have distorted somewhat the true form, and this may account for the varied relation of length to width in these measurements. We may roughly speak of the larger bodies as from 0.3 to 0.4mm (0.012 to 0.016 inch) in diameter.

A superficial microscopic examination showed at once that they were sporozoa. At the time of their discovery I had just found the

intraglobular parasite of Texas fever. Some relation between these intestinal parasites and those of the blood in Texas fever seemed at least a possibility. I therefore gave them as much attention as I could in connection with the somewhat trying microscopic work demanded by the Texas-fever investigations. Subsequently I found the same bodies in cattle from a Washington abattoir. Their relation to the Texas-fever micro-parasite could not, therefore, be upheld any longer, even if their morphology warranted such an assumption, which it does not.

The following observations were made exclusively on fresh material. They are incomplete in many ways, since only the reproductive stage has been seen. They will be continued as opportunity presents, since this group of organisms demands the attention not only of the zoölogist, but of the pathologist as well.*

It has already been stated that the lower portion of the small intestine is the preferred seat of these sporozoa. A few have been detected in the cæcum. They are especially abundant in young animals, and these animals are the only ones which present a sufficient number of stages of the life history of the parasite for investigation. Older animals generally contain a few cysts in an advanced stage of development, but they do not repay examination. I am inclined to regard midsummer as the time when the parasite appears most abundantly, but I have not extended the observations sufficiently into other seasons to make any positive statements on this point.

1. Let us first take the largest cysts readily visible to the naked eye. When crushed under a cover glass in some physiological salt solution the cyst wall tears readily and appears as a very delicate membrane of a finely granular appearance, readily thrown into folds by the crushing. A double contour was not observed. The crushing forces out of the densely packed cyst an immense number of falciform bodies of the same form and size, showing no connection with one another. These swarm spores are from 10.5 to 12.6μ in length and slightly crescentic in outline (Fig. 2, *a*). The body is homogeneous in appearance. Any differentiation of the cell contents is not observable in this condition. The majority show distinct movements like those originally described by Eimer for the coccidia discovered by him in the mouse's intestine. One end of the crescent bends toward the body, the whole appearing in this condition like the figure 6, or like a hook, or even like the letter U. After remaining in this bent, tetanic state for one or two seconds, the crescent suddenly straightens out, and in some instances it was propelled forward, *i. e.*, in the direction of the other extremity, from one-half to twice its whole length (Fig. 3, *a*). Whether only one-half of the crescent is the contractile portion, or whether both share this function, I am not prepared to say. The power to move had not been lost by

*Dr. C. W. Stiles, zoölogist of the Bureau, will continue the study of this organism.

a stay in the refrigerator for twenty-four hours (55° to 60° F.). The path of the crescent is usually a slightly curved line, corresponding to the curvature of the crescent in its straightened position. In straightening out it may be swung around by this force so as to describe a complete circle. In some cysts forms like *b*, Fig. 3, may be observed moving around this middle point as a pivot without straightening out. In one cyst bent forms with a central knob were observed, which, without showing any change of form, moved quite a distance. In one case the path described by the crescent might be compared to a loop. It will be seen from these illustrations that the motion of these bodies, which can be studied to great advantage in this sporozoön, is by no means easily explained, and will repay careful investigation.

The simple crescents retain their form well in normal salt solution, but when this is replaced by distilled water they speedily assume the spherical form and are disintegrated.

2. Cysts containing independent crescents of the simpler form described (Fig. 2, *a*) are perhaps the most commonly met with. Yet they are not the only ones, for after more or less investigation I found a bewildering variety of cyst contents which will require much patient observation to elucidate their relationship to one another. Not infrequently I found large cysts containing, besides the crescents described, peculiar modifications of the latter, as is shown in Fig. 2. These modifications appear as a central, round finely granular body, about 3μ in diameter attached to the middle point of a thread-like body, the whole being as long as the crescent described (12μ). These sometimes predominate in a cyst, sometimes they are present in small numbers mingled with the others. I have not observed any motion of the slender filiform bodies. It is safe to assume that they are crescents which have undergone further development within the cyst, and that Fig. 2 represents a related series of forms from *a* to *e*, inclusive.

3. In the case of calves minute cysts are frequently detected with a hand lens. Such cysts vary much in the morphology of their contents, and give us some idea of the way in which such a large number of swarm spores may be formed in a single cyst. When such cysts are examined entire under a low power a slight differentiation in the contents may be occasionally detected in the form of minute darker specks (Fig. 1). When the cyst is crushed the outpouring free crescents are accompanied by peculiar agglomerations of crescents. A small spherical body containing a variable number of refracting granules is everywhere beset with the homogeneous crescents first described. Each crescent is attached with one extremity to the periphery of the sphere, and extends radially or somewhat tangentially outwards. Some of these colonies, as they roll out of the crushed cyst, give the impression of sunflowers, the central sphere representing the disk and the crescents the ray flowers (Fig. 4, *a*, *b*). In several cases I was fortunate enough to find a still younger stage of the same structures, as is shown

in Fig. 5. The periphery of a rather large sphere loosely filled with refracting granules is beset with quite short conical appendages, the base resting on the sphere.

In some cysts nearly all the crescents are attached in the manner described. The central sphere varies much in size. The largest may be 15 μ in diameter, the smallest but 2 μ. The crescents may be so densely packed around the central body as to obscure it almost entirely. In the case of the smaller spheres there may appear but one to three attached crescents.

4. In the contents of the cysts described there are frequently found among the crescents a variable number of round bodies or spheres, from 4 to 9 μ in diameter, which are filled with minute refrangent particles, and to whose periphery no crescents in any stage of development are seen attached.

5. A few cysts, not more than half the diameter of those described, contained only masses of strongly refracting bodies 1 to 2 μ in diameter.

From this cursory description it will be seen that only a fragment of the life history of the sporozoön has come under my observation. The youngest stage, its penetration into the tissue of the villus, its true situation there, whether originally as an intracellular or an extracellular parasite, and the form and characters of the adult parasite still need elucidation. That portion of the life history found by me seems to be concerned mainly with the production of a large number of swarm spores. How these are formed in such abundance is shown at least partially by the observations recorded. A large number of centers or foci seem to be present throughout the cyst, from the periphery of which the crescents are produced. The previous history of these foci is not cleared up, but I have seen in various cysts forms roughly reproduced in Fig. 6, which lead me to believe, at least provisionally, that these foci are formed by a simple transformation of some of the crescents into spheres which subsequently produce, peripherally, a second generation of the same elements. In this way we may have several generations of crescents in the same cyst, and the enormous numbers may be the offspring of a few original crescents. As to the correctness of this theory future observations must decide. In several cysts I observed forms resembling *g*, in Fig. 2. The crescent was bent upon itself and apparently within a cyst of its own. Further observations will be necessary to determine whether such endogenous formation of crescents actually takes place. If so, it may represent crescents of a different functional order from those described, and it may explain the presence of the peculiar bent or U-shaped forms (Fig. 2, *f*; Fig. 3, *b*, *c*).

The functions of the swarm spores probably is to keep up the autoinfection of the intestinal mucosa. The rupture of one of the large cysts sends into the intestine a great number of such bodies, each of which is capable of forming a new focus of infection. The different stages in which these cysts are found lends color to this view. It is probable that

there exists also a more resistant stage whose function it is to keep up the life of the parasite outside of the animal body. I am inclined to look upon the tailed modifications of the crescents (Fig. 2, *c*, *d*, *e*), found only in the largest and oldest cysts, as such a resistant stage (*Dauerform*, *Dauerzustand*). The recent investigations of R. Pfeiffer* on *Coccidium oviforme*, and of Schuberg† on *Eimeria falciformis*, have shown that for these coccidia two types of reproduction may perpetuate the species, which in the case of *C. oviforme* were formerly looked upon as two distinct species. It is not to be denied therefore that the supposition concerning a more resistant stage of the crescents given above may be erroneous, and that future investigations may reveal a quite different type of reproduction.

The evidence thus adduced is not sufficient to bring this sporozoön into any of the known groups, but it at least indicates that it does not belong to the Coccidia, and that it has characters which might bring it within the range of the Sarcosporidiæ.

To give an adequate idea of the various stages in which the cyst contents are found, I will cite a few illustrations from my notes:

October 11, 1889.—Several cysts, from small intestine of healthy steer slaughtered for beef, contain only the homogeneous crescents (Fig. 2, *a*), many in motion.

One cyst contains a variety of forms, from the simple crescent to the knobbed forms (Fig. 2, *a* to *f*, inclusive).

September 20, 1889.—Case of Texas fever. One cyst contains mainly forms shown in Fig. 6.

September 20, 1889.—One cyst, from intestine of healthy steer, contains only such forms as are shown in Fig. 4. All crescents are attached to spherical bodies of various dimensions (2 μ to 15 μ).

A second cyst from the same animal contains free crescents in motion and a small number of round granular bodies (Fig. 5, *a*).

August 20, 1890.—One cyst, from a calf which succumbed to Texas fever, contains an immense number of refracting granules, 1 μ diameter, and many spherical bodies with small crescents attached (Fig. 5). Whether the free granules were originally contained in small cysts and freed by crushing was not determinable.

A second cyst contains, besides a few sunflower forms (Fig. 4), an immense number of free crescents.

A third very small cyst contains only refracting granules, 1 to 2 μ in diameter.

September 22, 1890.—One cyst, from a calf killed after a mild attack of Texas fever, contains motile crescents and granular spheres (4 to 9 μ in diameter).

A second cyst contains only knobbed crescents in the stage of *b*, Fig. 2.

A third small cyst contains almost exclusively immature attached crescents (Fig. 5, *b*, *c*, *d*, *e*).

August 11, 1890.—One cyst, from a healthy slaughtered steer, contains mainly tailed (Fig. 2, *d*, *e*, *f*) forms and a few crescents.

A second cyst contains (1) homogeneous crescents in motion; (2) tailed or knobbed forms; (3) many crescents bent in form of the letter U (Fig. 2, *f*); (4) crescents attached to a central sphere; (5) forms like Fig. 2, *g*.

A third cyst contains crescents only. These are of the usual form or doubled up (U-shaped) or partly extended. The U-shaped forms are quite abundant.

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*Untersuchungen über Protozoen, Heft. 1, 1892.

†Ueber Coccidien des Mäusedarmes. Sitzungsber. d. Würzb. phys-med. Gesellschaft, 1892.

DESCRIPTION OF PLATE I.

Illustrations, somewhat diagrammatic, of the various observed forms of the sporozoön. Fig. 1 is magnified about 80 diameters, the rest, 1,000 diameters.

Fig. 1. Cyst as seen under a low power of the microscope. The darker spots represent the forms shown in Fig. 4. The remainder of the cyst is filled with free crescents.

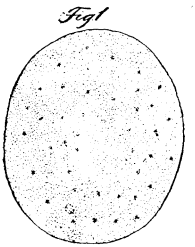
Fig. 2. Crescents of various forms.

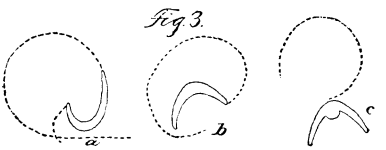
Fig. 3. Crescents in motion. The dotted lines indicate approximately the paths.

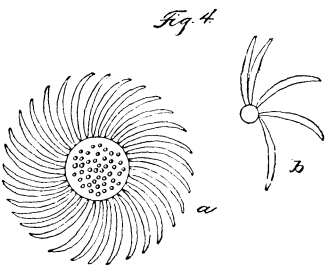
Fig. 4. Spheres containing granules, with periphery beset with nearly mature crescents.

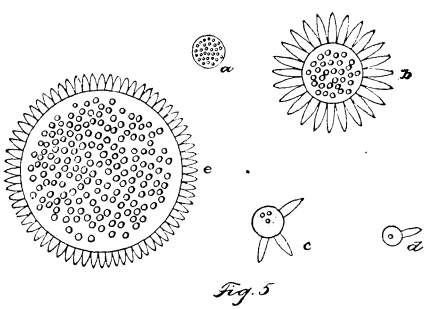
Fig. 5*a*. Simple sphere containing granules; *b*, *c*, *d*, and *e*, spheres with periphery beset with immature crescents.

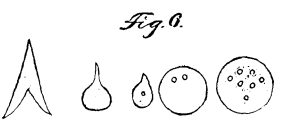
Fig. 6. (Hypothetical), transformation of a crescent into a sphere.











SPOROZOÖN IN THE INTESTINAL VILLI OF CATTLE.

NOTES ON PARASITES—18: ON THE PRESENCE OF SARCOSPORIDIA IN BIRDS.

By C. W. STILES.

[Plates II and III.]

The first reference I have been able to find in regard to Sarcosporidia in birds is an article by J. Kühn.* This paper is unfortunately not at my disposal, but it is evident from the statements of later authors that Kühn found the parasites in chickens.

Riley† examined a duck whose muscles were infested with parasites one-fourth to one-fifth of an inch long; they resembled grains of wheat and contained a viscid yellowish substance. Riley compared them to pork measles (*Cysticercus cellulosæ*) and was under the impression that they represented the larval stage of some worm which would reach maturity in the intestine of man. Upon seeing one of the specimens I describe below, Riley stated to me that it was undoubtedly identical with his duck measles.

Bélanger‡ presented before the Société d'Histoire Naturelle de Quibec a case of parasitism in *Spatula clypeata* Boie. Small "white worms" were found by thousands in the muscles. They measured ½–1 line long, and appeared to belong "à quelques genres voisins des trichines, bien qu'ils ne fussent pas enroulés, ni renfermés dans des enveloppes." This reference, to which Prof. Riley called my attention, probably refers to the same parasite described below from the same host.

Rivolta§ states:

Gli otricelli psorospermici dei gallinacei, di cui fanno menzione alcuni trattastisti (Röll ecc.) altro non sono che gli otricelli del Miescher o del Renay, che non si devono confondere con i psorospermi, propriamente detti, dei polli.

On page 398, *l. c.*, in discussing *Enterite psorospermica*, he adds:

Alle pareti poi dell' intestino si scorgono, situati all' incerca nel sottomucoso, dei punti bianchicci sparsi qua et là; quasi tutti della grandezza di un seme di papavero o di un semplice punto. Questi punti bianchicci non sono formati da colonie di psorospermi ma bensì da otricelli del Miescher o del Renay pieni di navicelle.

Rivolta also gives two figures of the parasites.

*Mittheilungen d. landwirthsch. Instit. zu Halle, 1865, p. 68.

†Walsh and Riley. A Measly Wild Duck. The American Entomologist, 1869, I, p. 89.

‡Le Naturaliste Canadien, Tome II, Mai, 1870, p. 188.

§Dei parassiti vegetali, Torino, 1873, p. 390. Taf. III, figs. 52, 74.

Leidy* describes some cysts found in the interstices of the muscles of *Anas boschas* L. They were oval, white, 2 to 4 mm. long, 0.7 mm. thick, and contained myriads of "fusiform corpuscles" 17μ long, resembling minute pseudo-navicellæ. He adds that "though the Mallard is not a fish eater, the bird may have become infected by eating diseased fish." Thus it is clear that Leidy was inclined to look upon these parasites as identical with the Myxosporidia.

Leuckart† simply states in regard to Sarcosporidia in relation to birds, "selbst das Haushuhn bleibt davon nicht verschont."

Bütschli‡ refers to the observations by Kühn and Rivolta as follows:

Kühn§ fand sie auch bei Hühnern und Rivolta hat auf das Vorkommen ähnlicher Parasiten in der Submucosa des Darmes mehrerer Vögel (der Haushühner, Schwarzamsel (*Turdus merula*), des Rabens, etc., aufmerksam gemacht und es scheint auch, dass diesselben mit Recht den parasitischen Schläuchen der Säugethiere an die Seite gesetzt werden.

Zürn‖ states that Sarcosporidia (*Psorospermienschläuche*) have been reported in the muscles, kidneys, and intestinal walls of various birds. He himself had never found them. The parasites are in some cases microscopic, in others visible to the naked eye; they may be 1 or several mm. long and are surrounded by a resistant but porous membrane. The crescents are 0.01 mm. long and contain one or more vacuoles, together with granules. They are not scattered regularly through the entire cyst, but collected together in balls.

Barrows¶ found a *Parula pitiayumi* (Vieill.) whose muscles were parasitized, and upon examining one of my specimens Mr. Barrows said that the parasite appeared identical with those he found, so far as this could be determined without a microscopical investigation. His description reads:

One specimen (*i. e., Parula pitiayumi*) seemed to be abnormal in coloring, showing many white feathers in the forehead, and upon skinning the flesh was found thickly spotted with oval, white lumps about the size of the egg of the common blow-fly. These were most numerous toward the surface of the pectoral muscles, but occurred also deeply imbedded in their substance as well as in the muscles of wings and legs. It was not practicable to examine their structure with the microscope until the next day, when decomposition was so far advanced that little could be made out. In all probability, however, they were the encysted larvæ of some parasitic worm, though whether they had anything to do with the abnormal plumage is an open question.

Barrows' description renders it almost certain that this was a case of sarcosporidiosis, although it is, of course, impossible to determine

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* *Psorosperms* of Mallard Duck. Proc. Acad. of Science, Phila., 1875, XXVIII, p. 125.

† Die Parasiten des Menschen, 2. Aufl., 1879, I, p. 254.

‡ Protozoa, I, p. 605. Bronn's Klassen und Ordnungen des Thier-Reichs, 1880–'82.

§ Sopra i vajuolo dei Columbi e dei Polli. Giornale di Anatomia, Fisiologia e Patalogia degli Animali, 1874, VI, p. 257. (N. B.—The pagination of this journal is incorrect. It should read, p. 321.)

‖ Die Krankheiten des Hausgeflügels, 1882, p. 146.

¶ Walter B. Barrows. Birds of the Lower Uruguay. Bull. of the Nuttall Ornithological Club, VIII, 1883, p. 87.

whether the parasites were identical with any of the forms described below or not. From the size of the animal ("the size of the eggs of the common blow-fly", *Lucilia cæsar*) it is probable that the parasites belonged to the genus *Balbiania*.

R. Blanchard* does not believe that the parasites mentioned by Rivolta in chickens are Sarcosporidia, but rather that they belong to *Eimeria*.

Mr. Haines, artist, upon seeing my specimens, said that he had noticed this same parasitized condition of the pectoral muscles in chickens upon several occasions, while Prof. C. Hart Merriam, of this Department, tells me that he has not infrequently seen similar bodies in the muscles of various birds.

I have mentioned above all the references I have been able to trace here at Washington of parasitic Sarcosporidia in birds.

While this list may not be entirely complete, it is quite evident that this parasitism, although by no means rare, has attracted very little attention, and that we have as yet no exact description of any one species of Sarcosporidia in birds. On this account I have deemed it advisable to place on record several cases of this kind which have recently come under my observation, and to give a more or less minute description of the animals, so far as the condition of the material at hand will permit.

1. The U. S. National Museum, through Prof. Riley, has recently referred to this Bureau a portion of the pectoral muscles of the Shoveller or Spoon-billed Duck (*Spatula clypeata*), taken by Walter Brett at Clear Lake, California.

2. From the U. S. Army Medical Museum I have a specimen of parasitized muscle labelled "*Cysticerci* (?) from the Shovel-bill Duck." (*Sp. clypeata*).

3. In our own collection in the Bureau of Animal Industry we possess a specimen labelled "From ducks. Prof. Lüger, Minnesota, said that falciform bodies escaped from cysts like *Balbiania gigantea*."

4. In Leidy's collection of parasites, which the University of Pennsylvania has recently sent to me for revision, I have found a bottle containing a portion of muscle labelled "Oval, smooth bodies, no limbs. In muscles of Mallard. *Anas boschas*. Dr. E. Coues. Ex. Jan. 26, 1890."

5. Finally, a second bottle referred to me by the U. S. National Museum contains the legs of a Grosbeak (*Habia ludoviciana*). The label reads "Explorations in Manitoba, Walter Brett. *Habia ludoviciana* ♂ (1166). Riding Mountain, June 16, 1892."

An examination of the muscle in all cases shows that it contains numerous elongated cysts lying parallel to the muscle-fibers and perfectly visible to the naked eye. The parasites found in specimens 1 to

*Note sur les Sarcosporidies et sur un Essai de Classification de ces Sporozoaires. Bull. d. l. Soc. Zool. d. France, 1885.

4 are identical, while two different forms of Sarcosporidia are found in specimen 5.

1. Balbiania Rileyi sp. n., 1893.

[Plate II, Figs. 1-5.]

The parasites in specimen 1 are in a tolerably good state of preservation, while specimens 2 to 4 are not so well preserved. They measure 1 mm. to 6 mm. in length, 0.48 mm. in diameter in their middle. They are rather fusiform, but the ends are somewhat blunt. Coloring liquids differentiate the body into two distinct portions—a center core, which has the general form of the entire body and is about 0.16 mm. broad in its greatest diameter, and a peripheral portion having about the same thickness as the center core. In some specimens the peripheral zone is even broader than the central core. The meshes of the central core may either be empty or contain a granular matter which does not take stain; while the meshes of the peripheral layer are filled with numerous lancet or falciform bodies, to the staining of which is due the differentiation of the entire body into the center core and peripheral layer.

The falciform bodies measure 12 to 14μ long by 2μ broad. They are slightly more pointed at one end than at the other. They contain a distinct oblong nucleus (about 2μ) which stains (Figs. 4, 5) very clearly with methyl blue or acid carmine. On unstained preparations (Fig. 5, *b*) the nucleus appears as a bright refrangent body. Its position is not constant. The rest of the falciform body is granular. Occasionally a small but indistinct vacuole is noticed.

The outer covering of the Sarcosporidium is a very thin (2μ) cuticular membrane. In this no striation could be discovered. The entire inner portion of the animal is a meshwork or stroma, in the interstices of which lie the numerous falciform bodies. Although there is no regular arrangement or form to the meshes of this stroma, in general they radiate from the center to the periphery and are longer (*i. e.*, in a radial direction) than broad (*i. e.*, in a tangential direction).

The entire stroma can be separated from the cuticle and pressed out, but I was unable to isolate the meshes one from another. Treated with a strong solution of caustic potash the contents of the meshes were destroyed. The walls, however, remained intact. In nearly all cases the wall between any two adjacent meshes appeared to be a simple membrane, common to both lumina, but in one or two cases the walls split longitudinally for a short distance, thus giving the impression that each lumen possessed its own wall and that the adjacent walls of the two meshes were very intimately united. In places where three meshes came together at one point there was frequently a triangular space between the three membranes.

The parasites lie between the muscle-fibers, surrounded by connective tissue. A very careful examination of a number of microtome sections failed to show any young stages inside of the muscle-fibers.

It is evident from the above that this parasite is very closely allied to

Balbiania gigantea Railliet, and *B. mucosa* R. Bl. Placing it in the genus *Balbiania*, I propose the specific name *B. Rileyi*, dedicating the species to my colleague, Prof. C. V. Riley, Entomologist of the United States Department of Agriculture, as he is evidently the first scientist who examined this parasite.

Diagnosis.—*B. Rileyi*, sp. n., 1893. 1–6 mm. long by 0.48 mm. broad; rather fusiform, ends not sharply pointed. Cuticle not striated, about 2μ thick. Center core does not color and does not contain falciform bodies. Peripheral zone as broad as central core (0.16 : 0.16 mm.) or even broader; colors in various liquids (acid carmine or methyl blue), and contains numerous falciform bodies. Form of meshes irregular, but elongated radially. Falciform bodies 12 to 14μ long, more pointed at one extremity than at the other; contain a very distinct nucleus (2μ) which stains clearly in acid carmine or methyl blue, and which contains several chromatophil granules; vacuole quite indistinct.

Habitat.—Intermuscular connective tissue of ducks (the Shoveller or Shovel-bill Duck or Spoon-bill Duck (*Spatula clypeata*) and the Mallard Duck or Tame Duck (*Anas boschas*).

Development unknown.

Geographical distribution.—North America. (?) Philadelphia, Pa. (Coues-Leidy); St. Louis, Mo. (Riley); Clear Lake, Cal. (Brett); Minnesota (Lüger); Quebec (Bélanger).

Type material deposited in the U. S. National Museum, in the Bureau of Animal Industry, and in Collection of Stiles, Washington, D. C. Specimens are also to be found in the Army Medical Museum, Washington, D. C., and in Collection of Leidy, University of Pennsylvania, Philadelphia, Pa.

In conclusion, although "measly duck" is not very appetizing in appearance, there are no grounds for believing that it is dangerous to man.

Specimen 5, mentioned above, contained, as already stated, two different forms of Sarcosporidia, which could easily be seen through the skin of the animal. The difference between the two forms, however, could be recognized only in microscopic preparations. One form was thicker, the cuticle unstriated so far as I could discover, a central non-staining core was present, and the parasites were intermuscular. The other form was much thinner, possessed a transversely striated cuticle, contained no central non-coloring core, and the parasites were intramuscular, *i. e.*, inside of the muscular fibers.

Blanchard (*l. c.*) has proposed the following provisional classification of the Sarcosporidia:

I. Family *Miescheridæ*:
  Situated in the striated muscles, enveloping membrane—
    a. Thin and structureless ..... *Miescheria.*
    b. Thick and traversed by fine canals ..... *Sarcocystis.*
II. Family *Balbianidæ*:
  Situated in the connective tissue, enveloping membrane thin and without structure ..... *Balbiania.*

I have already stated * that I agree with those authors who consider that the membrane of *Sarcocystis* is finely striated, instead of possessing pores—there can be absolutely no doubt in regard to this point in *S. tenella* of sheep—so that according to Blanchard's classification, if we substitute "finely striated" in place of "traversed by fine canals," the thicker specimens mentioned above will belong to the genus *Balbiania*, while the thinner form must be placed in the genus *Sarcocystis*.

Finding, as I have, the two forms lying side by side in the same host, there are two alternatives open to us to explain their presence:

(1) Either we have here a case of infection by two Sarcosporidia of different genera; or (2) *Sarcocystis* is only a younger intramuscular stage of the intermuscular *Balbiania*.

Although the second explanation is very tempting to us, yet having but a portion of a single animal infested with the two forms now under consideration, it would be too hazardous for me to state that this is the correct explanation, and to attempt to prove it, especially as the following facts can not be considered confirmatory of this view:

(1) In the case of *B. Rileyi*, I have found no intramuscular stage.

(2) In the horse, sheep, and hogs, it is a very common occurrence to find only intramuscular Sarcosporidia.

Thus, for the present, I feel compelled to place the two forms now under consideration in two separate genera, *Sarcocystis* and *Balbiania*, respectively, although I will give to both the same specific name, so that they may more easily be united in case they prove to be two stages of the same species.

2. Balbiania falcatula sp. n., 1893.

[Plate III, Figs. 1-2.]

This form appears quite distinct from *B. Rileyi*, in that it is longer in proportion to its breadth; the central non-coloring core is much wider in proportion to the peripheral coloring portion; the meshes in the center core are smaller and more regular, while the meshes of the peripheral zone generally have a more regular outline; the falciform bodies are only about half as large (6μ) as those of *B. Rileyi*, and generally more curved; they possess a very prominent nucleus, but no vacuole or portion resembling a vacuole could be discovered, except that the middle portion stained more lightly than the ends.

Specific diagnosis.—*B. falcatula* sp. n., 1893. The parasites measure 1.3 mm. to 3.2 mm. long by 0.4 mm. to 0.42 mm. broad; fusiform; cuticle not striated, about 2μ thick. Center core does not color, and contains no falciform bodies; meshes quite regular, 8μ to 12μ. Peripheral zone much narrower than centre core (0.096 mm. : 0.224 mm.), colors in various liquids (acid carmine, for instance) and contains numerous

* Review of recent publications in Medical Zoölogy. The Journal of Comparative Medicine and Veterinary Archives, 1891, p. 693.

lciform bodies measuring 5μ to 6μ long by 2μ broad; the latter curved, ntaining a distinct nucleus (1.5μ) generally situated at one end; no acuole visible (alcohol material).

Habitat.—Intermuscular connective tissue of the Rose-breasted rosbeak (*Habia ludoviciana*).

Development unknown.

Geographical distribution.—North America, Manitoba (Brett).

Type material deposited in the U. S. National Museum, in the Bu- au of Animal Industry and in Collection of Stiles, Washington, D. C.

3. Sarcocystis falcatula sp. n., 1893.

[Plate III, Fig. 8.]

These parasites lie within the sarcolemma; they measure on an aver- e 2.4 mm. long by 0.152 mm. broad; the ends are pointed, the membrane μ thick) is finely striated; no non-coloring central core could be dis- nguished. At the extremities the divisions or meshes in which the lciform bodies lie are more rounded and distinct, but near the middle the body it is almost impossible to recognize these divisions. The lciform bodies I could measure only on microtome sections. They peared about 6μ long.

Specific diagnosis.—*S. falcatula* sp. n., 1893. Body 2.4 mm. long by 152 mm. thick; fusiform; cuticle finely striated; meshes at extremities unded and distinct, in middle portion indistinct; falciform bodies 6μ ng.

Habitat.—Muscle-fibers of *Habia ludoviciana.*

Life history unknown.

Geographical distribution.—North America, Manitoba (Brett).

Type material deposited in the U. S. National Museum, in the Bu- au of Animal Industry, and in Collection of Stiles, Washington, D. C.

Species inquirendæ.

4. Sarcosporidia of chickens found by Kühn (1867), at Halle, Ger- ny, and by Haines (cited above) in Maryland, U. S. A.

5. Sarcosporidia—probably *Balbiania* sp. found by Barrows in *Parula iayumi*, at Concepcion del Uruguay, province of Entre Rios, Argen- e Republic, South America.

6. ? Sarcosporidia described by Rivolta (1874) from chickens, *Turdus rula* and crows (cited from Bütschli).

DESCRIPTION OF PLATE II.

Fig. 1. A portion of the pectoral muscles of a duck (Measly duck).

Fig. 2. Longitudinal section of *Balbiania Rileyi* (greatly enlarged).

Fig. 3. Transverse section of *Balbiania Rileyi* (greatly enlarged); *ct*, connectiv tissue cyst, with numerous nuclei; *cu*, cuticle of the parasite; *m*, sections of muscl

Fig. 4. Microtome section of meshes containing falciform bodies, greatly enlarge

Fig. 5. Falciform bodies of *Balbiania Rileyi; a*, stained, showing nucleus an vacuole; *b*, unstained.

All drawings by Mr. Haines, artist, detailed to this Bureau, drawn either fro nature or after sketches by author.

Fig. 2

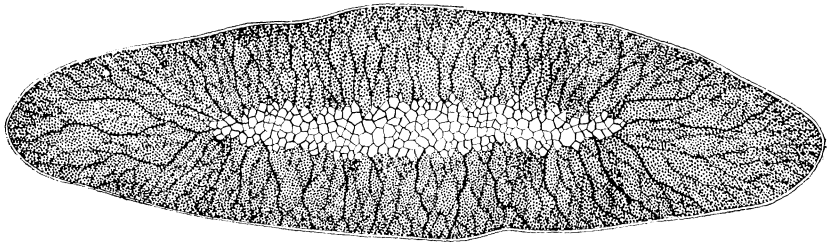


Fig. 1

Fig 5

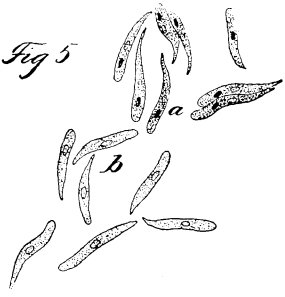
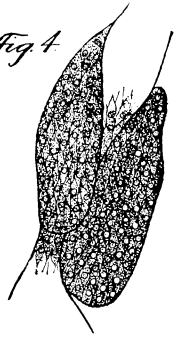


Fig. 4



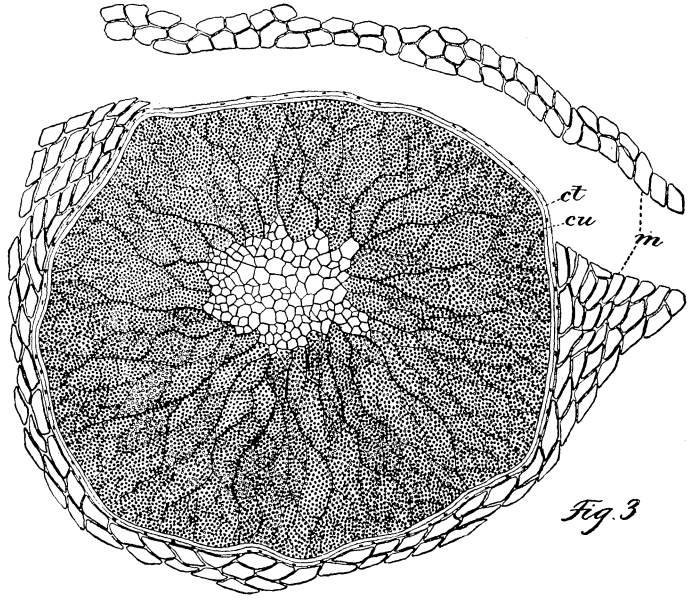


Fig. 3

BALBIANIA RILEYI sp. n.

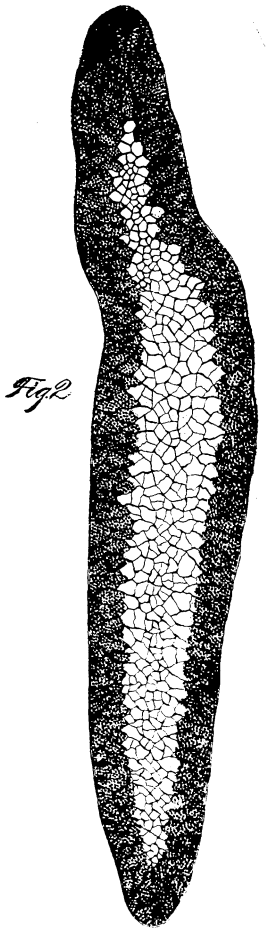
DESCRIPTION OF PLATE III.

Fig. 1. Falciform bodies of *Balbiania falcatula.*

Fig. 2. *Balbiania falcatula,* longitudinal section.

Fig. 3. *Sarcocystis falcatula,* longitudinal section. It will be noticed that the ends of the parasite are included in a muscle-fiber which has lost part of its transverse striation. In the specimen here drawn the muscle-fiber could not be traced the entire length of the parasite.

Fig. 3*a*. A portion of the striated cuticle, seen on section.



BALBIANIA FALCATULA sp. n., and SARCOCYSTIS FALCATULA sp. n.